Dynamics of Pyrogenic Organic Matter: Physical, Chemical and Biological Transformation and Stabilization Processes in the Soil

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Summary

Pyrogenic organic matter (PyOM) is formed during the incomplete combustion of biomass. PyOM constitutes an important carbon (C) pool in soil and sediments and has significant role in global processes, including soil C storage, soil fertility, atmospheric reactions and earth's radiative budget. In recent years, PyOM has gained importance as C sink in soil due to its resistance to degradation. However, there is wide knowledge gap in the understanding of PyOM C dynamics and large uncertainties in its effect on terrestrial and global C cycle.

This thesis contributes to the understanding of PyOM dynamics and compares it with its initial biomass (in present study, *Pinus ponderosa* wood). The main objective of this thesis was to estimate the turnover time of PyOM in soil, quantitatively assess C and N fluxes from PyOM and wood, determine chemical changes in PyOM with time and investigate main stabilization mechanisms for PyOM in the terrestrial system. Other important objectives were to assess the effect of increased N on both PyOM-C and wood-C dynamics as well as quantitatively and qualitatively measure the effect of PyOM and wood input on microbial community structure and activity.

Within the scope of this PhD work a meta-analysis was performed using data from 16 published studies (n = 54) on PyOM C degradation to estimate the turnover time using two modelling approaches. To address the questions raised in this PhD work, a field study was conducted using dual labeled ^{13}C and ^{15}N enriched PyOM and *Pinus ponderosa* wood and their fate in soil was quantified over ten months, *in situ*. The solubility of fresh PyOM-C and 10 y aged PyOM was tested in a batch experiment. Molecular community structure was studied using pyrosequensing approach. Furthermore, a novel approach to measure isotopic signature of molecular markers for PyOM-C was developed.

Based on the meta-analysis on PyOM C degradation studies, the estimated turnover time ranged from a decadal to centennial time scale, varying with initial biomass type, pyrolysis temperature, and incubation or field study. The result suggested that PyOM degrades in the soil. However, there is no single rate of decomposition of PyOM C in all soils and conditions and the interpretation is limited due to lack of long-term field studies (>5 y). The field study using dual isotope label substrates revealed that there was no apparent loss of PyOM C although there was a small but significant change in the overall chemical structure of PyOM. In contrast, 48% of the initial amount of wood C was lost after 10 months. The result corresponds to annual to decadal turnover times for wood C. PyOM C moved vertically downward in the soil profile with 3-4% of PyOM C recovered below the application depth (0-5 cm). One mechanism explaining the transport of PyOM could be leaching via solubilization. However, fresh PyOM showed only a small fraction was soluble (<0.3% of the total mass) that eventually increased with ageing as observed in the

batch experiment testing solubilization of fresh and aged (10 y) PyOM. Together, these findings suggest that loss of PyOM C via leaching could be an important loss mechanism on a long-term.

Fractionating soil into aggregate and density fractions quantified for the first time that one-third of the PyOM C was incorporated into physically protected fractions *in situ* within a year. This highlighted that PyOM persistence in the soil need not be solely due to its chemical structure. In contrast, wood C showed significantly higher interaction with soil mineral phases than PyOM and was recovered mainly in aggregates or associated with soil minerals (≥60% of total wood C). PyOM primed the loss of native soil C and in this study it was revealed for the first time that particularly the free light fraction (up to 12%) gets affected due to priming. However, loss of native soil C in wood-amended soil was not significant.

The effect of added N treatment on the different parameters considered in this study was observed specially in wood-amended soil. Moreover, added N decreased the growth of organisms involved in N cycling. However, these changes are limited and affect the C and N cycles only on the margin.

Furthermore, the isotopic analysis of PyOM molecular markers was developed in collaboration with University of California, Davis. The ionic properties of benzene polycarboxylic acids (BPCA) facilitated individual BPCAs separation by anion-exchange chromatography followed by its measurement using ion exchange chromatography-isotope-ratio mass spectrometry (IEC-IRMS). The accuracy and precision of this method were evaluated and was observed to be a suitable method to measure range of sample types from PyOM to soils at both natural and artificial ^{13}C abundance.

After ten months, the effect of wood and PyOM amendment change neither the microbial biomass nor its activity (expressed as enzyme activity). Moreover, at the phylum level, there was no change in the structure of soil bacterial community. Nevertheless, family of phylum *Actinobacteria* and *Bacteriodetes* increased in their relative abundance while *Deltaproteobacteria* decreased in PyOM-amended soil. Notably, wood-amended soil under added N treatments resulted in the change in microbial community structure with significant changes in the relative abundance of family *Actinobacteria* and *Betaproteobacteria*. The result suggested that the shift in soil bacterial community due to PyOM is evident only at fine levels of taxonomic resolution.

In summary, this thesis quantified various fluxes, stabilizing mechanisms and physico-chemical to biological effect of both PyOM and wood C in the soil, *in situ*. However, in the changing climate and with course of time these mechanisms could alter and future work requires focus on the effect of environmental variables for instance, temperature and moisture, and ageing of PyOM on the turnover time and dynamics of PyOM.

Zusammensfassung

Pyrogene organische Substanz (*engl.* pyrogenic organic matter, PyOM) ist das Produkt der unvollständigen Verbrennung von Biomasse. PyOM stellt einen wichtigen Bestandteil des Kohlenstoff- (C-) Vorrats in Böden und Sedimenten dar und spielt eine entscheidende Rolle bei globalen Prozessen, wie der Speicherung von C in Böden, der Bodenfruchtbarkeit, als organisches Adsorbens, bei atmospherischen Prozessen und bei der Strahlungsbilanz der Erde. In den vergangenen Jahren ist die Bedeutung von PyOM als Kohlenstoffsenke im Boden diskutiert worden, da PyOM sich relativ inert gegenüber Abbauprozessen verhält. Allerdings sind die Kohlenstoffumsätze von PyOM und deren Effekte auf den terrestrischen und globalen Kohlenstoffkreislauf mit grossen Unsicherheiten behaftet und liegen bislang teils im Verborgenen.

Diese Arbeit soll zum Verständnis der Umsätze von PyOM beitragen und stellt einen Vergleich mit den Umsätzen des Ausgangsmaterials an (in dieser Studie gemahlenes Holz aus *Pinus ponderosa*). Das Hauptziel der Arbeit besteht darin, die Umsatzzeiten von PyOM im Boden besser abzuschätzen, die mit PyOM und dem Ausgangsmaterial assoziierten C und Stickstoff (N) Flüsse quantitativ zu erfassen, die chemischen Veränderungen im Laufe der Zeit zu bestimmen und die wichtigsten Stabilisierungsmechanismen für PyOM zu untersuchen. Einen weiteren wichtigen Aspekt stellt die Erforschung der Rolle von erhöhter N-Verfügbarkeit auf die Umsätze von PyOM und Holz dar. Des Weiteren sollen die Effekte von PyOM und Holz auf die Zusammensetzung und Aktivität der mikrobiellen Gemeinschaft qualitativ und quantitativ erfasst werden.

Im Rahmen dieser Doktorarbeit wurde eine Metaanalyse aus Daten von 16 veröffentlichten Studien zum Abbau von PyOM-C erstellt (n=54). Die Ergebnisse aus der Metaanalyse wurden verwendet, um die Umsatzzeit abzuschätzen, wobei zwei verschiedene Modellansätze zum Einsatz kamen. Es wurde eine Feldstudie mit ^{13}C und ^{15}N markiertem PyOM und Holz durchgeführt und der Verbleib im Boden über einen Zeitraum von 10 Monaten beobachtet. Darüber hinaus wurde eine neue Methode zur Bestimmung der isotopischen C-Signatur von molekularen PyOM-Markern entwickelt.

Die Metaanalyse der PyOM-Abbaustudien ergab eine geschätzte C-Umsatzzeit in der Grössenordnung von Jahrzehnten bis Jahrhunderten, wobei das Ergebnis vor allem durch die Ausgangsbiomasse, die Pyrolysetemperatur und von dem Experimentaufbau (Feldstudie oder Laborinkubation) beeinflusst wurde. Es wurde deutlich, dass PyOM im Boden abgebaut wird. Allerdings kann kein allgemein gültiger Wert für die Abbaurate von PyOM für die verschiedenen Bodentypen und Bedingungen angegeben werden. Zudem ist die Aussagekraft der Ergebnisse durch den Mangel an Langzeitstudien (länger als 5 Jahre) zum PyOM-Abbau begrenzt. Die Feldstudie mit der C- und N- Isotopenmarkierung der beiden Substrate ergab keinen

messbaren Austrag von PyOM-C aus dem Bodenprofil. Hingegen war eine kleine, aber signifikante Veränderung in der chemischen Struktur von PyOM erkennbar. Im Gegensatz dazu waren nach 10 Monaten die Verluste von C aus Holz mit 48% viel höher, was eine Umsatzzeit innerhalb von Jahren bis Jahrzehnten erwarten lässt. PyOM wurde im Profil nach unten verlagert, wobei 3-4% unterhalb der Ausbringtiefe (0-5 cm) wiedergefunden wurden. Ein möglicher Mechanismus für die Verlagerung ist die Auswaschung von wasserlöslichen Bestandteilen von PyOM. Allerdings ist die Wasserlöslichkeit von frischer PyOM mit < 0,3% äusserst gering. Diese kann aber bei Alterung der PyOM deutlich zunehmen, wie aus einem Extraktionsversuch mit frischer und gealterter PyOM (10 Jahre) bekannt ist. Insgesamt deuten diese Ergebnisse darauf hin, dass Auswaschung ein wichtiger Prozess bei der langfristigen Verlagerung von PyOM sein kann.

Zum ersten Mal konnte mittels einer kombinierten Dichte- und Grössenfraktionierung gezeigt werden, dass ein Drittel des PyOM-C bereits nach einem Jahr *in situ* in physikalisch geschützte Aggregate übergegangen war. Dies macht deutlich, dass die Stabilität von PyOM im Boden nicht allein durch die chemische Struktur bestimmt wird. Verglichen mit PyOM zeigte Holz eine deutlich grössere Interaktion mit der Mineralmatrix des Bodens, wobei der grösste Teil in Aggregaten und an Mineraloberflächen gebunden gefunden wurde (≥ 60% des Holz-Kohlenstoffs). Die Zugabe von PyOM förderte den Abbau des Bodenkohlenstoffs ("priming"), und zum ersten Mal konnte gezeigt werden, dass davon besonders die partikuläre, organische Fraktion betroffen war (bis zu 12 %). Hingegen waren Verluste des Bodenkohlenstoffs bei der Zugabe von Holz gegenüber der Kontrolle nicht signifikant erhöht.

Der Effekt der erhöhten Zugabe von N zeigte sich bei den in dieser Studie untersuchten Parametern vor allem bei den mit Holz versetzten Böden. Des Weiteren reduzierte die Gabe von N das Wachstum von Organismen, die am N-Kreislauf beteiligt sind. Allerdings sind diese Effekte sehr begrenzt und verändern den C- und N-Kreislauf nur geringfügig.

Des Weiteren wurde eine Isotopenanalysemethode von molekularen PyOM-Markern in Zusammenarbeit mit der University of California, Davis entwickelt. Die Ioneneigenschaften der Benzolpolycarbonsäuren ermöglichten deren Trennung mittels Anionen-Austauschchromatographie (IEC) mit anschliessender Bestimmung durch Isotopenverhältnis Massenspektrometrie (IRMS). Die Genauigkeit und die Präzision dieser Methode wurden überprüft. Die Methode ist geeignet um verschiedene Proben von PyOM, von Holzkohlen bis hin zu Bodenproben, sowohl mit natürlichem als auch mit erhöhtem ^{13}C Gehalt zu messen.

Nach zehn Monaten konnte weder bei der Zugabe von Holz noch bei der Zugabe von PyOM eine Veränderung in der mikrobiellen Biomasse oder ihrer Aktivität (gemessen als Enzymaktivität) festgestellt werden. Auch in der Bodenbakterien-Gemeinschaft konnte keine Veränderung auf der Stammebene beobachtet werden. In den mit PyOM versetzten Böden nahmen die Familien der Stämme

Actinobacteria) und *Bacteriodetes* in ihrer relative Häufigkeit zu, wohingegen bei *Deltaproteobacteria* eine Abnahme zu beobachten war. Mit Holz versetzte Böden bei zusätzlicher Gabe von N wiesen eine veränderte Zusammensetzung der mikrobiellen Gemeinschaft auf, wobei sich die relative Häufigkeit der Familien *Actinobacteria* und *Betaproteobacteria* veränderte. Die Ergebnisse zeigen, dass eine Veränderung in der Gemeinschaft der Bodenbakterien nach der Zugabe von PyOM nur bei hoher Auflösung der Taxonomie erkennbar wird.

Im Rahmen dieser Arbeit konnten verschiedene Flüsse, Stabilisierungsmechanismen, sowie physikalisch-chemische und biologische Effekte von PyOM und Holz *in situ* ermittelt und quantifiziert werden. Allerdings können sich diese Mechanismen unter veränderten Klimabedingungen und im Laufe der Zeit anders darstellen. Zukünftige Arbeiten sollten daher besonderes Augenmerk auf die Bedeutung der Umweltfaktoren, wie etwa Temperatur und Feuchtigkeit, sowie auf Veränderungen der Abbaudynamik im Zusammenhang mit der Alterung von PyOM legen und diese bei der Berechnung der Umsatzzeiten von PyOM berücksichtigen.

Contents

Abbreviations

ATP	Adenosine triphosphate
BG	β-1,4 glucosidase
BPCA	Benzene polycarboxylic Acids
B3CA, B4CA, B5CA, B6CA	Benzenecarboxylic acids with 3, 4, 5 or 6 carboxylic groups, respectively
CBH	β-cellobiohydrolase
DF	Dense fraction
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
fLF	Free light fraction
GC-FID	Gas chromatography-flame ionization detector
Gt	Gigaton (10^{12} kg)
HPLC-IRMS	High-performance liquid chromatography–isotope-ratio mass spectrometry
IEC-IRMS	Ion-exchange chromatography–isotope-ratio mass spectrometry
NAG	β-N-acetylglucosaminidase
oLF	Occluded light fraction
PE	Priming effect
Pg	Petagram (10^{12} kg)
PyOM	Pyrogenic organic matter
SMB	Soil microbial biomass
SOC	Soil organic carbon
SOM	Soil organic matter
Tg	Teragram (10^{9} kg)

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Part A – Synopsis

1 Introduction

Scientists have scoured ecosystems from the ocean's depths to the highest mountain peaks searching for signals of global change. But only recently has this attention extended under the Earth's surface to the soil.
Richard D. Bardgett, 2011

On a global scale, soils contain thrice as much carbon (C) (2500 Gt in soil with 1500 Gt in the upper 100 cm depth as organic C) as the atmosphere (760 Gt), and four folds the C in vegetation (560 Gt) constituting an important C reservoir (Gt = 10^9 tons) (Batjes, 1996, Jansson *et al.*, 2010, Lal, 2004). The ever-increasing human interventions in the Earth system have greatly accelerated emissions of carbon dioxide (CO_2) into the atmosphere putting Earth's ecosystems on a trajectory towards rapid climate change. CO_2 emissions are majorly derived from fossil fuels and biomass burning and contribute to the greenhouse effect and therefore to climate change. Article 3.4 of the Kyoto Protocol on reducing the net emission of greenhouse gases recognizes the importance of C in the soil as a store, source and potential sink of CO_2, in addition to supporting functions of the aboveground biomass (Sombroek *et al.*, 2003). The changing climate scenarios and global changes could modify biogeochemical cycling of C between the atmosphere, the vegetation and soil organic matter (SOM). Therefore, soil C pool is expected to become an increasingly important determinant of global C cycle in the near future.

One of the important contributors to the soil C pool is pyrogenic organic matter (PyOM), an incomplete combustion residue of biomass burning (Goldberg, 1985). PyOM is present virtually in all soils and sediments (Schmidt & Noack, 2000). It is a soil C pool that is spatially variable on both regional and global scale (Figure 1). The significance of PyOM is manifold including soil C storage and dynamics (Sombroek *et al.*, 1993, Sombroek *et al.*, 2003), soil fertility (Chan *et al.*, 2007, Lehmann *et al.*, 2003, Liang *et al.*, 2006), adsorbent for range of contaminants (Beesley & Marmiroli, 2011, Yang & Sheng, 2003), long-term C sink in ocean sediments (Masiello & Druffel, 1998, Middelburg *et al.*, 1999), dissolved organic carbon (DOC) formation (Dittmar *et al.*, 2012, Ziolkowski & Druffel, 2010), atmospheric chemistry (Ramanathan & Carmichael, 2008) and the Earth's radiative budget (Hansen & Nazarenko, 2004, McConnell *et al.*, 2007). Moreover, future climate predictions indicate increase in the frequency and intensity of wildfires (Flannigan *et al.*, 2006, Moritz *et al.*, 2012) that could modify the PyOM pools in the environment, especially in the soil. However, there is a wide gap in the knowledge on PyOM C dynamics and large uncertainties exist for its effect on the terrestrial and global C cycle. Therefore, there is a need to better understand the dynamics of PyOM in soils.

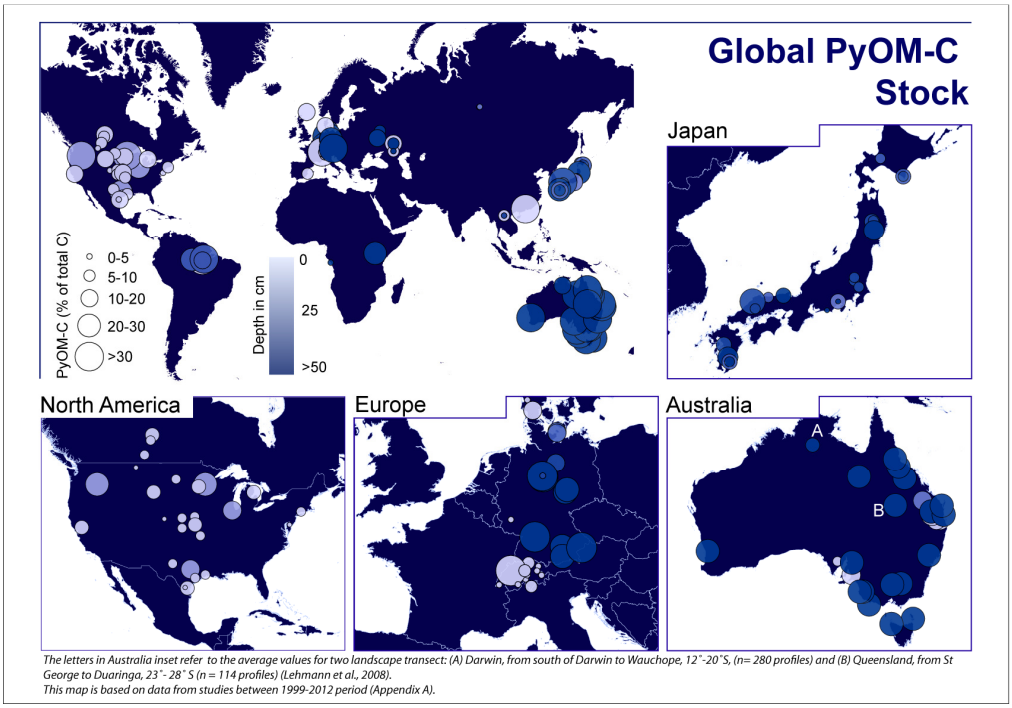

Figure 1: *PyOM C stock (as % of total C) in different geographical locations based on data collected from previously published studies (n=200). The circles in the map indicate the locations where different studies estimated the stock of PyOM C. The size of the circles corresponds to the amount of PyOM C expressed as the percentage of total organic C with the biggest size for values >30% of total C. The increasing color intensity of the circles denotes increase in the soil depth (cm). The details of the data used for this map are summarized in Appendix (Table A1).*

1.1 Pyrogenic organic matter: definition, characteristics and significance

The carbon cycle has a very long equilibrium time. The consequences of actions taken now will persist for many centuries.
Scholes 1999

PyOM refers to the solid residue of biomass after its incomplete combustion (also referred as black carbon, charcoal, soot, pyrogenic carbon, biochar). PyOM does not have a well-defined chemical structure and is usually conceptualized as "combustion continuum" and constitute C forms of varying hybridization states, aromaticity and degree of condensation (Hammes *et al.*, 2006, Keiluweit *et al.*, 2010, Preston & Schmidt, 2006, Schmidt & Noack, 2000). The formation of PyOM is purely terrestrial in origin. It occurs via two fundamentally different ways: (i) recondensation of volatiles to form soot and (ii) the solid residue commonly referred to as charcoal (Schmidt & Noack, 2000). The heating of biomass causes loss of H, C and O in the form of H_2O, CO_2, CO, CH_4 and other simple volatiles, resulting in the decrease of H/C and O/C molar ratios of the PyOM produced compared to its initial biomass

(Almendros *et al.*, 2003, Hammes *et al.*, 2006, Keiluweit *et al.*, 2010). This is due to gradual rearrangement and formation of aromatic ring structures followed by a progressive condensation of smaller aromatic units into larger conjugated sheets (Baldock & Smernik, 2002, Knicker *et al.*, 1996, Preston & Schmidt, 2006, Shafizadeh & Sekiguchi, 1983). All PyOM, therefore, is characterized by the presence of polycondensed aromatic networks. However, PyOM is highly variable in its physical architecture and molecular composition depending on its initial biomass and pyrolysis conditions resulting in widely differing dynamics and functions in soils and sediments (Keiluweit *et al.*, 2010).

PyOM (in the form of charcoal) has long been used as a source of paleoenvironment information and study of fire frequency during the Holocene (Cohen-Ofri *et al.*, 2006) but in recent years it has received considerable interest because of its potential relevance in the C cycle and climate change mitigation. A special report of the IPCC on renewable energy source and climate change mitigation highlighted the importance of those strategies that could sequester C from active cycles (IPCC, 2011). The addition of PyOM to the soil would also constitute one such strategy if C contained in PyOM was resistant to degradation after its formation and incorporation in the soil. The view that PyOM forms a slow cycling C pool in soils comes from the fact that PyOM contains some of the most resistant organic compounds (e.g. PAHs, condensed aromatic structures) known on Earth's surface (Masiello 2004). It is often the only identifiable remnant of prehistoric human settlements (Cohen-Ofri et al. 2006), preserved in geological samples or strata (Forbes et al., 2006), archaeological sites (Schmid et al., 2002; Glaser, 2007), and old anthropogenic soils (Glaser et al., 2000; Knicker, 2011; Glaser and Birk, 2012). PyOM is resistant to chemical oxidants (Skjemstad et al., 1996) and has contributed to the oldest soil organic carbon (SOC) pools in some Australian soils (Krull et al., 2006). Therefore, production of PyOM, in combination with its storage in the soil, has been suggested as one possible means of reducing the atmospheric CO_2 concentration (Fowles, 2007, Glaser *et al.*, 2001, Laird, 2008, Lehmann *et al.*, 2006, Sombroek *et al.*, 1993).

1.2 PyOM in the soil: the "Black Box" of the soil carbon pool.

> *Permanence is the probability that stored carbon will remain out of the atmosphere. If the conditions that created the sink are not maintained, carbon in the form of plant biomass or soil organic matter is liable to return to the atmosphere, either abruptly (for example, through fires, storms, or pest outbreaks) or more gradually through respiration.*
> Scholes and Noble, 2001

PyOM was considered to constitute a small fraction of the C globally cycled and was majorly considered as a C sink (Forbes *et al.*, 2006, Marris, 2006, Seiler & Crutzen, 1980). The persistence of PyOM in the environment is however contradicted by recent experimental studies that observed onset of transformation and partial mineralization of PyOM over weeks and yearly timescales (Baldock & Smernik, 2002, Hamer *et al.*, 2004, Hilscher & Knicker, 2011b, Kuzyakov *et al.*, 2009, Santos

et al., 2012) and significant losses in field studies over time (Bird *et al.*, 1999, Hammes *et al.*, 2008, Nguyen *et al.*, 2008). We, however, lack sufficient quantitative understanding of biogeochemical cycling of PyOM in the environment. PyOM literally represents a "black box" of the soil C pool of which processes involved in its formation, loss and stabilization are not well understood (Knicker, 2011). At present, the estimation of PyOM production, its fluxes and pools are limited by its high variability in the amount as well as chemical and molecular forms produced during wildfires. It is further limited due to range of methods used to determine PyOM component in post fire residues. To understand the role of PyOM in global C cycling and assess sustainability of any C sequestration attempt involving PyOM in the long term requires research that would minimize the uncertainties and discrepancies regarding estimates of PyOM fluxes between the atmosphere, terrestrial system and oceans.

1.2.1 Fluxes of PyOM: Loss from the terrestrial system

Overall, PyOM production is estimated to be <3% of the C that is consumed during fire events, with >80% (40 to 241 Tg C y^{-1}) deposited on the soil near the site of production and 5–6 Tg C y^{-1} released into the atmosphere (Forbes *et al.*, 2006). From soils, PyOM is transported due to erosion (Chaplot *et al.*, 2005, Rumpel *et al.*, 2006b) and/or by fluvial mechanisms (Elmquist *et al.*, 2008, Kuhlbusch, 1998, Masiello & Druffel, 2001, Mitra *et al.*, 2002, Schmidt & Noack, 2000) to oceans (1.4-5.4 Tg C y^{-1} to coastal ocean and 2.4–4.8 Tg C y^{-1} to open ocean) where they get deposited in ocean sediments. Mass balance calculation based on the production of PyOM C and the amount lost as deposition in ocean sediments showed that soil PyOM-C pool should be 350–1900 Pg, or 25–125% of the total soil organic C pool (Masiello & Druffel, 2003). However, PyOM constitutes upto 45% of the total soil organic carbon (SOC) (Schmidt *et al.*, 1999) and therefore other loss processes exists for PyOM. Recently, PyOM has been shown to degrade in natural environment and physical, chemical, or biological mechanisms has been identified that could cause this (Fig. 2).

The mechanisms involved in the degradation and stabilization of PyOM in soils has been shown in Fig. 2. PyOM produced during wildfires are either lost from the terrestrial system by various mechanisms or gets incorporated, stabilized and subsequently accumulates in the soil. Loss of PyOM from the soil surface occurs via erosion (Rumpel *et al.*, 2006b) which is enhanced by pulverization of PyOM at the surface by soil processes, for instance, Shrink-Swell dynamics (Gouveia & Pessenda, 2000), frequent freeze-thaw cycle especially in areas with steep temperature gradients (Carcaillet, 2001) or by fungal hyphae penetrating soiled surface exerting a significant force (Hammes & Schmidt, 2009). However, fragmentation of PyOM once in the soil is not an important loss mechanism (Thery-Parisot *et al.*, 2010). PyOM is also lost from surface layer during subsequent fires (Czimczik *et al.*, 2005). From surface, PyOM is incorporated and translocated to the deeper soil horizons (Carcaillet & Richard, 2000, Dai *et al.*, 2005, Rumpel *et al.*, 2006a, Skjemstad *et al.*,

1999) by, soil faunal mixing (Carcaillet, 2001, Topoliantz & Ponge, 2003) or action of roots (Zackrisson *et al.*, 1996).

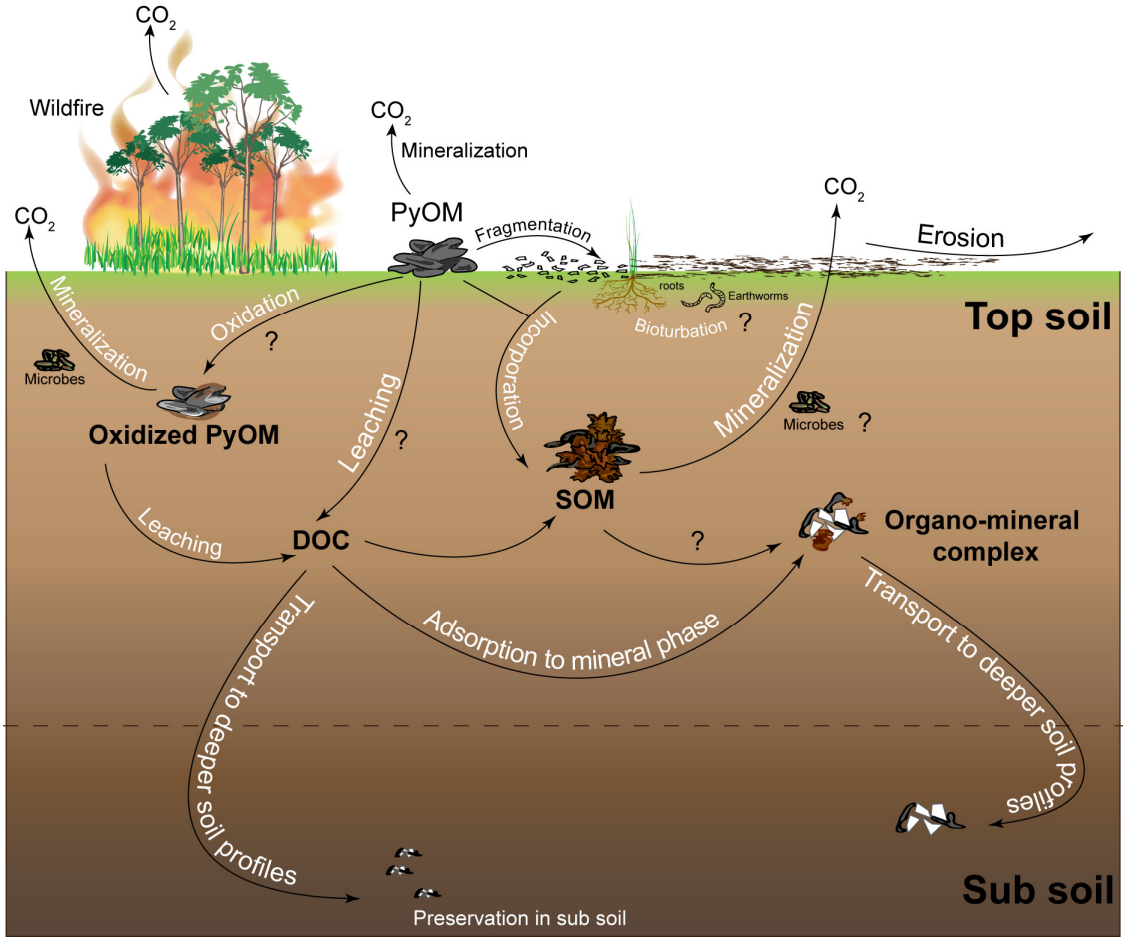

Figure 2: *Schematic representation of dynamics of PyOM in terrestrial system. The arrows indicate the processes and mechanism for losses and stabilization of PyOM C in soils. The question marks indicate the questions addressed in this thesis.*

Biological degradation of PyOM has also been reported in several studies, for instance, slow oxidation of amorphous carbon (Potter, 1908), oxidation of labeled graphite in a non-sterile soil system (Shneour, 1966), production of large amount of humic acids on exposure to microbial enzymes (Hockaday *et al.*, 2006) and more recently in incubation studies on degradation of PyOM (Brodowski, 2005, Hamer *et al.*, 2004, Hilscher & Knicker, 2011b, Zimmerman, 2010). However, microorganisms responsible to degrade and utilize PyOM as C source remain unknown. In nature, beside biotic and mechanical loss processes, PyOM could also degrade chemically. Abiotic oxidation of PyOM has been observed in incubation studies (Cheng *et al.*, 2006, Nguyen & Lehmann, 2009, Nguyen *et al.*, 2010, Zimmerman, 2010). At present, the understanding of PyOM losses and mineralization rates are majorly derived from laboratory incubation studies under controlled conditions. However,

very little is known about the dynamics of PyOM under field conditions. Moreover, the effect of changing climate scenarios on PyOM degradation rates remains unknown.

1.2.2 PyOM preservation and stabilization in the soil

The longevity of PyOM as observed by its ^{14}C radiocarbon ages and its detection in archeological sites are contradicted by the recent estimates of turnover time of PyOM. The question is why PyOM persist in some soils for thousands of years? In past, PyOM stability was only attributed to its intrinsic chemical structure (Baldock *et al.*, 2004, Schmidt & Noack, 2000). However, PyOM stability has been shown to vary in different environmental settings. It has been suggested that long-term persistence of PyOM could depend on its inaccessibility to microorganisms (Schmidt *et al.*, 2011, von Lützow *et al.*, 2006), such as through physical protection and interactions with soil minerals (Brodowski *et al.*, 2006, Cheng & Lehmann, 2009, Cheng *et al.*, 2006, Cusack *et al.*, 2012, Glaser *et al.*, 2000, Vasilyeva *et al.*, 2011). Types of soil has also been postulated to play an important role in PyOM preservation (Czimczik & Masiello, 2007), for instance, soils rich in calcium content have a higher ability to hold PyOM via Ca^{2+} bridging (Clough & Skjemstad, 2000), volcanic ash soils could stabilize PyOM via Al and Fe oxyhydroxides (Golchin *et al.*, 1997) or soils with reactive clays (Smectite) retained more PyOM than with less reactive clays (Kaolinite) (Skjemstad *et al.*, 2001). The absence or presence of biological capability and capacity of the soil to degrade PyOM (for e.g. enzymes required for the degradation, microorganisms capable of producing these enzymes) also influences the stability of PyOM in the soil (Baldock *et al.*, 2004). The influence of these possible stabilization processes on the turnover time of PyOM in the soil and the factors that control them are fairly unknown.

1.3 Interactions between PyOM and the soil organic matter

The C cycle on the land we study will affect C flows beyond its fence; and flows beyond the fence will alter the C cycle within.
Janzen, 2004

The incorporation of PyOM in the soil alters the quantity and quality of SOM. The concentration of aromatic C has been found to increase with the addition of PyOM to soils (Haumaier & Zech, 1995, Knicker *et al.*, 2005a, Knicker *et al.*, 2005b). Several studies showed a relationship between soil dark color and PyOM content (Knicker *et al.*, 2006, Schmidt *et al.*, 1999). The size of PyOM C pool is closely correlated to the organic content of the soil (Cusack *et al.*, 2012, Glaser & Amelung, 2003). PyOM influenced the chemistry and ^{14}C age of SOC in Australian soils (Krull *et al.*, 2006). Recent observations also showed that the mineralization rates of the native SOM could be influenced by addition of PyOM to the mineral soil (i.e., priming effect, PE) (Wardle *et al.*, 2008). However, the direction of priming effect on the SOM due to

PyOM is under debate. PyOM has been shown to inhibit (negative priming) (Jones *et al.*, 2011), has no effect (Abiven & Andreoli, 2010, Hilscher *et al.*, 2009, Kuzyakov *et al.*, 2009, Santos *et al.*, 2012), or increase native SOM mineralization rates (positive priming) (Steinbeiss *et al.*, 2009, Zimmerman *et al.*, 2011). Moreover, in an incubation study using different types of PyOM, Zimmerman et al., (2011) observed change in PE from positive to negative with time. Recently, Woolf and Lehmann (2012) using a modeling approach estimated that the potential effect of an increased labile organic carbon decomposition rate on a long term SOC stock was negligible. Therefore, in order to understand the potential significance of C in the soil in the form of PyOM, its characteristics and dynamics should be compared to those of the remaining SOM, which accounts for majority of the C that exists in most soils. Moreover, it is important to understand the underlying physical, chemical and biological processes that could make the SOC stable or degradable in the presence of PyOM.

1.4 Effect of nitrogen deposition on PyOM dynamics

Linkages between the C and N cycles regulate the amounts, distributions, and turnover rates of C within many terrestrial ecosystems.
(Ågren *et al.*, 1991)

The carbon and nitrogen cycles are closely linked at all scales and interact in myriad ways and therefore cannot be looked separately (Norby, 1998). Over the past century, atmospheric deposition of reactive N has increased three to five folds (Denman *et al.*, 2007). Increase in N deposition, via agricultural fertilization and combustion of fossil fuel (Galloway *et al.*, 2008), has the potential to alter soil C storage via changes in the SOM decomposition and stabilization (Cleveland & Townsend, 2006, Cusack *et al.*, 2010, Swanston *et al.*, 2004, Townsend *et al.*, 1996, Waldrop *et al.*, 2004). N addition has decreased (Fog, 1988, Janssens *et al.*, 2010, Turunen *et al.*, 2004, Waldrop *et al.*, 2004), increased (Bragazza *et al.*, 2006, Mack *et al.*, 2004, Waldrop *et al.*, 2004), or had no effect on (Knorr *et al.*, 2005a) SOM decomposition rates. There are fewer studies on the effects of increased nitrogen on wood and PyOM decomposition. These studies observed increased (Allison *et al.*, 2009, Bebber *et al.*, 2011, Micks *et al.*, 2004, Wal *et al.*, 2007) or level (McColl & Powers, 1998) decomposition rates of wood, with no effect on PyOM (Santos *et al.*, 2012). Despite the growing interest in PyOM for C mitigation, little is known about the effect of N addition on PyOM dynamics and vice versa in the short or long term.

1.5 Challenges in the methodological assessment of PyOM

We have made the natural world our laboratory, but the experiment is inadvertent and thus not designed to yield easily decipherable results.
Lee R. Kump, 2002

The methodological assessment of PyOM is challenging due to its heterogeneous chemical structure and presence in various environmental compartments. Several methods exist to estimate the quantity and quality of PyOM. In a multi laboratory comparison study using seven of the most common methods, Hammes et al. (2007) observed that these methods showed large variation in the amount of PyOM C measured and each method was designed to measure a certain window of combustion continuum but none covering the whole spectrum. Among these methods, the benzene polycarboxylic acid (BPCA) molecular marker approach has been developed in the last decade as a reliable method that could quantify and simultaneously characterize the chemical structures of PyOM materials in soil matrices (Brodowski *et al.*, 2005b, Glaser *et al.*, 1998, Schneider *et al.*, 2010). Moreover, the BPCA method was shown to measure relevant fraction of PyOM C over broad temperature range including wildfires (Schneider, 2011).

Despite the diversity of methods available for quantifying PyOM, the methods available to quantitatively assess the decomposition of PyOM in laboratory or field experiments are similar to those used for the SOM, viz., (i) measurement of PyOM-derived CO_2 efflux from the soil (as difference from the control) (Baldock & Smernik, 2002, Hamer *et al.*, 2004, Liang *et al.*, 2008, Zimmerman, 2010) (ii) radiocarbon methods based on labeling of PyOM by ^{14}C (Kuzyakov *et al.*, 2009) and by (iii) tracing stable carbon isotopes (^{13}C) labeled PyOM (Hilscher *et al.*, 2009, Hilscher & Knicker, 2011a, Hilscher & Knicker, 2011b, Santos *et al.*, 2012) or using the difference in stable isotope signatures of C3 and C4 vegetated soils, for e.g. application of C3 plant biomass derived PyOM in a C4 vegetated soils (Major *et al.*, 2009a) . Another approach is based on loss of PyOM C in a chronosequence after the wildfire has ceased (Bird *et al.*, 1999, Hammes *et al.*, 2008, Nguyen *et al.*, 2008, Vasilyeva *et al.*, 2011).

One of the challenges is to combine the BPCA approach with stable and radiogenic C isotope tracers (i.e. ^{13}C or ^{14}C) to directly measure the movement, loss and transformation of PyOM, *in situ*.

2 Objectives

A large knowledge gap in the dynamics of PyOM in the terrestrial system is majorly due to lack in the understanding of the mechanisms responsible for its mineralization and stabilization. This thesis aims to improve the understanding of PyOM dynamics in a temperate forest soil and fill gaps in the estimation of its turnover time, fluxes and stabilization mechanisms in the terrestrial system. Most of the previous studies on this topic were performed in an incubation experiment and therefore cannot be extrapolated on an ecosystem scale with certainty. The nature of the questions being addressed in this work required the study in field-conditions taking into account the myriad interactions of different variables at an ecosystem scale.

1) What is the turnover time of PyOM-C in the soil and what factors control its residence time?

There are indications from earlier studies that PyOM degrades in soils (Spokas, 2010) but there is a huge variation in the turnover time estimated in these studies. The variation in the estimation of turnover time was partly explained by the differences in the methodological approach. Therefore, the aim of this work was to use one consistent approach within and across all studies to estimate turnover time of PyOM in the soil and highlight the factors that determines its stability in the soil.

***Hypothesis**:* PyOM undergoes slower degradation in comparison to its initial biomass and its turnover in thousands of years.

2) What is the decomposition dynamic of PyOM-C and wood-C in a temperate forest soil?

For evaluating the potential of PyOM as long-term C sinks in the soil as compared to its precursor biomass it is important to track the pathways of C and N during their decomposition in the soil. The use of highly labeled ^{13}C and ^{15}N PyOM and wood would enable us to improve the understanding of its dynamics in soils. To answer this question, the more specific objectives were to (i) determine recovery of PyOM and its precursor wood in the soil (0–15 cm) after 10 months; (ii) measure the vertical movement of PyOM and wood C and N in the soil profile; and (iii) determine the partitioning of PyOM and wood C and N within operational SOM fractions, to decipher the degree and main mechanism of stabilization in the soil.

Hypothesis: PyOM gets oxidized in the soil that eventually results in its (a) mobility and (b) interaction with mineral phase.

3) Does benzenepolycarboxylic acid (BPCA) molecular marker signature of PyOM changes with time during its degradation in the soil?

PyOM in the course of its degradation gets increasingly oxidized (Cheng *et al.*, 2008a, Cheng *et al.*, 2006). However, these changes could be restricted to the surface (Lehmann *et al.*, 2005) and not visible in the bulk (Schneider *et al.*, 2011). Using molecular marker approach, Hammes *et al.,*(2008) and Brodowski (2005) observed

changes in the relative contributions of the individual marker molecules of PyOM. The objective within this thesis was also to quantify the loss of BPCA-C yield and assess qualitative changes in PyOM with time.
Hypothesis: The labile forms of PyOM are preferentially degraded and lost while more stable forms become relatively enriched.

4) Does increased N treatment affects the decomposition dynamics of PyOM and wood in a temperate forest soil?
Several studies indicate that the decomposition rate of low quality litter (high lignin and low N content, e.g. wood) is negatively affected by N additions (Fog, 1988, Janssens *et al.*, 2010, Knorr *et al.*, 2005a) due to decrease in the activity of oxidative enzymes (Waldrop *et al.*, 2004). Similar effect of N addition is expected on PyOM, as it is known to degrade via biotic and/or abiotic oxidation (Cheng *et al.*, 2006). However, there is limited knowledge of N effect on PyOM dynamics.
Hypothesis: N additions to soils via atmospheric deposition may slow the decomposition of PyOM and wood in the soil.

5) How is microbial community affected by addition of wood and PyOM to soil?
Very little work has focused on the potential effects that PyOM and wood may have on soil microbial community structure and the biogeochemical processes that underpin many key ecosystem functions essential for soil and plant health. Recently, studies on PyOM-amended soil revealed that PyOM changes the community structure of microorganisms (Khodadad *et al.*, 2011, Santos *et al.*, 2012, Steinbeiss *et al.*, 2009) and could alter the microbial activity (Bailey *et al.*, 2011, Kolb *et al.*, 2009).
Hypothesis: Soil microbial biomass, activity and community structure is affected by PyOM amendment of the soil.

6) How could BPCA molecular marker be used for the isotopic characterization of PyOM-C in complex environmental matrices?
Among various techniques applied to measure the abundance of PyOM in different environmental samples, the molecular marker BPCA approach provide an important measure of PyOM dynamics by giving qualitative and quantitative information together. However, some BPCAs may be derived from non-PyOM sources (Glaser & Knorr, 2008, Haumaier, 2010). The use of stable isotope tracers (e.g. ^{13}C-PyOM) together with BPCAs would allow researchers to directly follow PyOM-derived BPCAs in environmental matrices. However, isotopic analysis of molecular markers for PyOM-C currently relies on gas chromatography–combustion–isotope-ratio mass spectrometry (GC-C-IRMS) of trimethylsilyl and methyl derivatives of BPCAs and therefore currently poses uncertainty due to external C added during sample separation. The objective was to develop a novel method to measure ^{13}C on BPCAs produced after PyOM oxidation.
Hypothesis: Ion exchange chromatography–isotope-ratio mass spectrometry (IEC-IRMS) may be a suitable alternative for the measurement of δ^{13}C-BPCA.

3 Summary of Materials and Methods

This section gives a brief summary of methodologies that were used to answer the research questions in this thesis. The approach to estimate the turnover time of PyOM (3.1), track the fate of C and N of PyOM in the forest soil (3.2, 3.3, 3.4, 3.5 and 3.6) and estimate the changes in microbial community and its activity due to PyOM input to the soil (3.7) has been discussed in the following sub-sections. This section does not introduce the methods but only discusses certain aspect of the method that was useful in achieving the objectives of this thesis.

3.1 Turnover time of PyOM and its estimation on compiled data

The "turnover time" is the average time that carbon resides in a (conceptual) SOM pool (Hakkenberg *et al.*, 2008). The decomposition of PyOM is assumed to follow first order kinetics. We therefore define the turnover time as the inverse of the decay rate constant, based on mono exponential decay model, Eq. 1. Therefore, it is the time required to lose e^{-1} of the initial amount (Fig. 3).

$$\text{Turnover time} = \frac{1}{k} = \frac{-t}{\left(ln(C_0 - C_t)\right)} \qquad (1)$$

where k is decay rate constant (y^{-1}), C_0 is the initial amount at time t = 0, C_t is the amount left after time t.

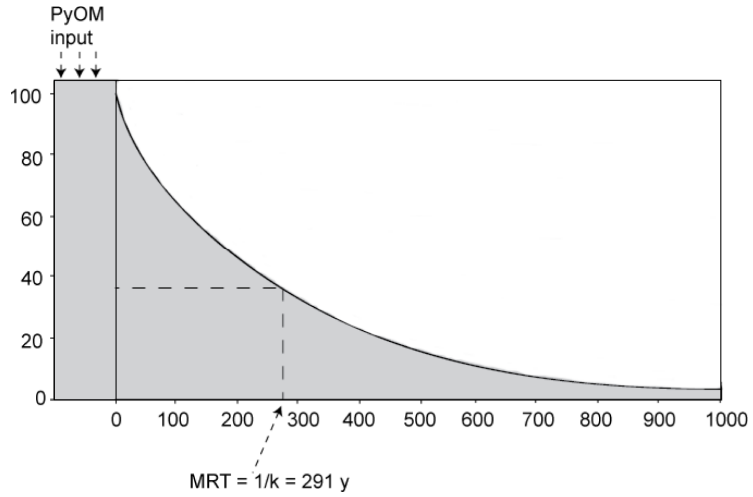

Figure 3: *The concept of turnover time based on mono exponential decay model. As an example the turnover time of PyOM is shown (Singh et al., 2012). It must be noted that the turnover time (in this example turnover time of PyOM is 1/k = 291 y) for mono exponential decay indicates when about 37% of the initial concentration is left (e^{-1} = 0.368) (Hofmann, 2009).*

The degradation rates and turnover time of PyOM in previous studies have been calculated using either one-pool decay model (Eq. 2)(Brodowski, 2005, Cheng *et al.*, 2008b, Hammes *et al.*, 2008, Nguyen *et al.*, 2008) or two-pool decay model (Eq. 3)(Hamer *et al.*, 2004, Hilscher *et al.*, 2009, Hilscher & Knicker, 2011b, Kuzyakov *et al.*, 2009, Major *et al.*, 2009a).

$$C_t = C_0 e^{-kt} \qquad (2)$$

where, C_t is the remaining stock after time t, C_0 is the initial stock of PyOM-C (at t = 0), and k is the decay rate (y^{-1}). The turnover time was calculated as $= 1/k$.

$$C_t = xe^{-k_{fast}t} + (1-x)e^{-k_{slow}t} \qquad (3)$$

where C_t is the remaining stock after time t; x is the proportion of initial stock in the fast-cycling PyOM C pool (at t = 0), C_{fast}; (1–x) is the proportion of the slow-cycling pool (at t = 0), C_{slow}; k_{fast} and k_{slow} are decay rate constants (y^{-1}).

To estimate the turnover time of PyOM in the soil, the data was compiled from published studies (n = 54 data sets from 16 studies, *Manuscript I*: Supplement Table A3) on PyOM C degradation. In *Manuscript I*, both these two models were used on the data compiled (n= 54) from broad set of published studies and the turnover time was calculated within and across all studies using this approach. There were some limitations and assumptions to this approach that has been detailed in the *Manuscript I*.

3.2 Study site and experimental design

The study site is located in a mixed beech temperate forest on the south-facing slope of Lägeren mountain (easternmost part of the Jura mountain range, 680 m a.s.l.), situated 20 km northwest of Zurich (47° 28' 40.8" N, 8° 21' 55.2" E), Switzerland. The experiment was set-up in a forest gap created by natural windthrow and subsequently mowed to maintain open conditions, giving it some similarity to a post-fire gap (Fig. 4). The soil at the site is classified as a Cambisol (F.A.O.-U.N.E.S.C.O., 1998). The chemical and physical properties of the soil (0-10 cm) are detailed in *Manuscript II* and summarized in Table 1.

Table 1: *Elemental content, stable isotope values and pH of the soil in the study area and organic input used in the field experiment.*

	pH	C	H	N	^{13}C	^{15}N	^{18}O
		g kg^{-1}			atom %		‰ (VSMOW*)
Soil	5.9	33.7	8.9	2.4	1.08	0.36	nd[1]
PyOM	7.5	779	34	7.1	2.05	4.3	13.2
Wood	nd[1]	494	66	4.3	2.03	4.2	26.0

*[1]nd = not determined, * Vienna Standard Mean Ocean Water, VSMOW*

Figure 4: *The wind-throw area in the Lägeren forest where the experiment was set up. The mean annual temperature is 8.4 ˚C and mean annual precipitation is 930 mm (Ruehr & Buchmann, 2010).*

We installed the cylindrical mesocosms (20 cm long and 10 cm diameter) in a randomized block design with three organic input treatments (pinewood, PyOM and no input as control) and two treatment levels of nitrogen [ambient N (N0) = 20 kg N y^{-1} ha^{-1} (Kloeti *et al.*, 1989) and increased N (N+) = +60 kg N y^{-1} ha^{-1}] with three field replicates (n=3) per treatment combination. Wood (primary stem biomass from two year old *Pinus ponderosa*) and PyOM (obtained by charring the wood at 450°C for 5 hours under N_2 flux) were highly and uniformly labeled with ^{13}C and ^{15}N (Santos *et al.*, 2012, Yarnes *et al.*, 2011). The use of stable isotopes allowed to track the dynamics of C or N in the soil and to assess PyOM and wood incorporation in microbial biomass or physically separated soil C pools. The chemical characteristics of both labeled wood (^{13}C = 2.05 atom% and ^{15}N = 4.3 atom%) and PyOM (^{13}C = 2.03 atom% and ^{15}N = 4.2 atom %) are described in Santos et al. (2012) and summarized in Table 1. ^{13}C and ^{15}N labeled wood and PyOM were applied to the mesocosms at a rate of 189 g C m^{-2} for wood or 397 g C m^{-2} for PyOM, at 1 cm soil depth. These application rates were based on previous estimates of PyOM inputs to the soil after a fire (Eckmeier *et al.*, 2007b). In the N+ treatment mesocosms, 11.4 mg of $NH_4^+NO_3^-$ dissolved in 10 ml of water was added biweekly (equivalent to 60 kg N ha^{-1} y^{-1}), while an equivalent amount of distilled water was added to N0 treatment mesocosms.

3.3 Soil sampling and analyses

We sampled the intact mesocosms (n=18) 10 months after the PyOM or wood addition to the soil mesocosms. The soil within the mesocosms was separated immediately into 0-5 cm, 5-10 cm and 10-15 cm depth (Fig. 5). Soil fauna, stones (>2 mm) and roots (>2 mm) were manually removed. Soil sub-samples were air-dried and ball-milled for physico-chemical analysis. An overview on the analytical methods with references that were applied in this thesis to answer the research questions is given in Table 2. The methodologies are detailed in *Manuscript II* and *Manuscript III*.

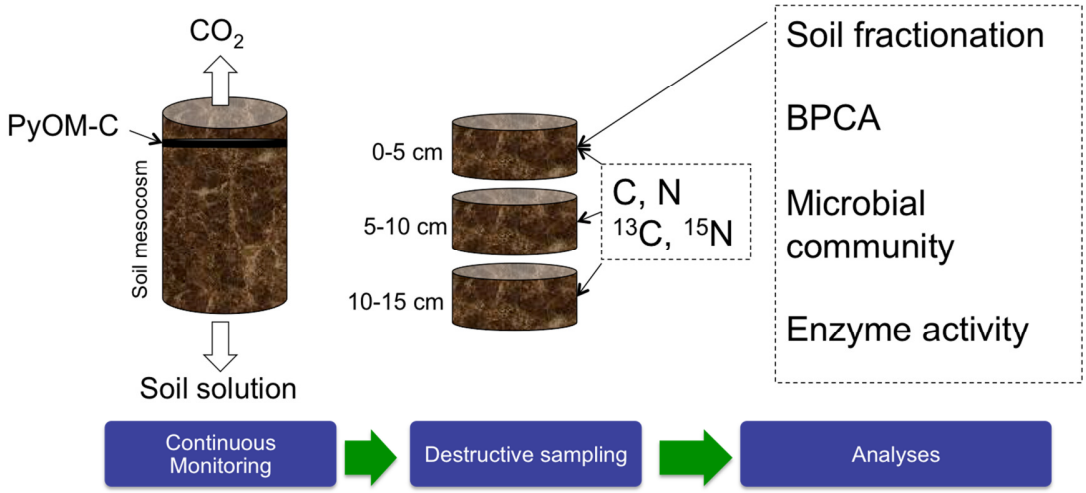

Figure 5: *Experimental set up in the field, sampling and corresponding analyses procedures.*

3.4 Stable isotope analyses

The application of ^{13}C and ^{15}N labeled organic inputs (wood and PyOM) in this thesis helped in clear separation of PyOM and wood derived C and N from native SOC and proved to be an extremely valuable and powerful tool for tracing the pathways of C and N in the terrestrial system. Fractions of labeled substrate C and N recovered in different soil depth and in the physically separated soil density fractions was calculated using a two end-member linear mixing model (Bernoux *et al.*, 1998) (as in Eq. 3 and Eq. 4, *Manuscript II*).

3.5 Density fractionation

To assess the distribution of PyOM and wood into discrete physical fractions as a means for identifying mechanisms like organo-mineral interactions, density fraction was used. In this study, the free light fraction (fLF) was separated using a density <1.6 g cm^{-3} (Cerli *et al.*, 2012, Glaser *et al.*, 2000) and the occluded light fraction (oLF) was separated by gentle ultrasonic dispersion (250 J ml^{-1}). The remaining soil fraction was considered as dense fraction (DF).

3.6 Benzenepolycarboxylic acid molecular marker method

This method is based on the production of a single aromatic ring with substituted carboxylic acids (3-6) (benzene polycarboxylic acids, BPCA) upon oxidation of PyOM under high temperature and pressure (Brodowski *et al.*, 2005b, Glaser *et al.*, 1998, Schneider *et al.*, 2010). The number of carboxylic acid groups on each BPCAs is a function of the number of aromatic carbons attached to it prior to oxidation. The quantitative distribution of the BPCAs formed is reflective of the original structure of the condensed aromatic material (Schneider, 2011, Ziolkowski & Druffel, 2009), Fig 6. This method provides both qualitative and quantitative information of PyOM. In this thesis, BPCA molecular marker method was therefore used to observe changes in the amount of PyOM derived C as well as overall molecular structure of the initial PyOM. This method was further used to elucidate the quality and quantity of PyOM-C leached out from both fresh and 10 y old PyOM used in a batch experiment (section 3.8).

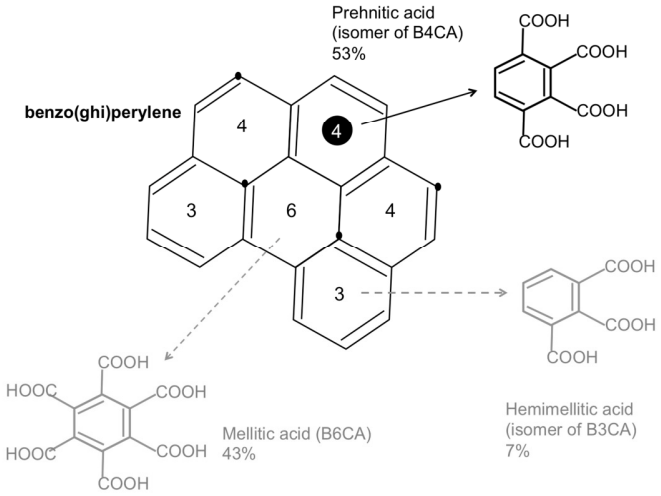

Figure 6: *Principle for the production of benzenepolycarboxylic acids (BPCA) explained using molecular structure of benzo[ghi]perylene (Schneider, 2011). An example here shows that black-circled ring on oxidation produced prehnitic acid (53% of total BPCA-C) where adjoining black dots positions are oxidized to carboxylic acids. The production of B6CA (40%) and B3CA (7%) is also possible but per molecule benzo[ghi]perylene only one BPCA molecule can be produced (shown in grey) (Schneider, 2011).*

For the measurement of stable isotope of BPCAs produced, a novel method was developed in this work using ion-exchange chromatography–isotope-ratio mass spectrometry (IEC–IRMS). The method development has been detailed in the *Manuscript IV*. Briefly, after BPCA production as described in the previous paragraph, individual BPCAs were separated at 30˚C on a Dionex IonPac® column

using a sodium hydroxide gradient. For six of the eight BPCAs, compounds were identified based upon retention times through the use of a prepared mixture of pure compounds. For two commercially unavailable B4CAs (1,2,3,4-B4CA and 1,2,3,5-B4CA), retention times and elution order were predicted from their published pKa values (Cerar & Podlipnik, 2008) and comparison with gas chromatography (GC) data from soil samples and standards with variable individual B4CA content. After separation, individual BPCAs are quantitatively oxidized to CO_2 by mixing it with sodium peroxidisulfate (0.8 M) and phosphoric acid (1.5 M) as it flows through an oxidation reactor heated at 99.9 ˚C. Each CO_2 peak is transferred from aqueous phase to helium carrier gas (2 ml min^{-1}) through a separation membrane and later transferred to an IRMS (Finnigan DeltaPlus Advantage, Thermo Electron, Bremen, Germany) through an open split. Post-analysis, the measurements were standardized with a prepared mixture of five BPCAs (BPCA-MIX) of varying concentration (50–500 ng C m^{-1} each).

3.7 Microbial response to PyOM input to the soil

The diverse soil microbial community present in a three-dimensional inorganic soil matrix mediates 80-90% of the processes in the soil (Nannipieri *et al.*, 2003). Therefore, one of the main objectives is to understand the role of microbes in PyOM decomposition. The response and the role of microbial community could be studied by estimating change in its (i) quantity (biomass), (ii) structure (diversity) and/or (iii) activity (enzymes). In this thesis, all three methods were used to decipher the role and effect by and on the soil microbes due to PyOM and wood input to the soil (Table 2).

Chloroform fumigation extraction was used to estimate microbial biomass C and N in the soil amended with wood/PyOM as well as in unamended control (Vance *et al.*, 1987). A conversion factor of 0.45 (K_c) (Wu *et al.*, 1990) and 0.68 (K_n) (Shen *et al.*, 1984) was applied for incomplete extraction for microbial C and N, respectively.

Pyrosequencing: In this thesis, pyrosequencing was employed to highlight the effect on microbial community structure due to added PyOM and wood to the soil. Pyrosequencing is a DNA sequencing technique that is based on detection of released pyrophosphate (PPi) during DNA synthesis (Ronaghi, 2001). The PPi is released during nucleic acid polymerization reaction as a result of nucleotide incorporation by polymerase. This is followed by conversion of PPi to ATP, by ATP sulfurylase, which subsequently provides the energy to luciferase to oxidize luciferin and generate light proportional to the number of incorporated nucleotides (Ronaghi *et al.*, 1998).

Enzyme assay was carried in order to gain insight into the effect of PyOM and wood amendment of soils on the microbial community function. Four enzymes were selected for this study: β-1,4 glucosidase (BG), β-N-acetylglucosaminidase (NAG), ,

β-cellobiohydrolase (CBH) and phenol oxidase. Fluorescence-based soil assays for BG, NAG and CBH were based on protocols using the following respective substrates: 4-methylumbelliferyl β-d-glucopyranoside (Sigma, M3633), 4-methylumbelliferyl-N-acetyl-β-d-glucosaminide (Sigma, M2133) and 4-Methylumbelliferyl β-D-cellobioside (Sigma, M6018). Colorimetric soil assays were conducted for phenol oxidase using the substrates 3,4-Dihydroxy-L-phenylalanine (Sigma, D9628). For the fluorescence-based assays, standard curves were developed and the enzyme activities were calculated against the appropriate curve.

3.8 Batch experiment to determine the solubility of PyOM in water

To estimate the amount of the potentially soluble and particulate fractions that could be released from a fresh PyOM (charcoal) and an aged PyOM, a batch experiment was conducted. Fresh PyOM was obtained by pyrolysing chestnut wood (*Castanea sativa*) at 450° C for 5 h under N_2 flux while aged PyOM was collected from an experimental forest fire where chestnut trees had been burned 10 years ago (Prometheus site, Ticino, Switzerland). The batch experiment involved shaking of 8 g of ground PyOM material in 100 ml of water for 6 hours and subsequently centrifuging at 4000 rpm. The soluble and colloidal fractions were collected by vacuum filtration over membrane filter papers of 0.45 µm (Whatman ME25, Schleicher & Schuell, Dassel, Germany) and 5 µm pore size (Whatman AE98), respectively. The collected fractions were subsequently freeze-dried and analyzed for BPCA using gas chromatography-flame ionization detector (GC-FID) as described above (section 3.6).

Table 2: *Summary of various analytical methods applied to track the pathways of C and N of PyOM and wood in the soil.*

Target	Method/equipment	References
C and N concentrations (*Manuscript II, IV* and *V*)	Elemental analysis using CHN elemental analyzer (EA 1108 Carlo Erba, Italy)	
^{13}C and ^{15}N of soils, wood, PyOM and density fractions (*Manuscript II* and *IV*)	Elemental analysis coupled to isotope ratio mass spectrometer (IRMS) (Delta S, Thermo Finnigan, USA)	
Density fractionation of soils (*Manuscript II*)	Using sodium polytungstate of density <1.6 g cm^{-3} and applying 250 J ml^{-} dispersive energy for occluded fractions	(Cerli *et al.*, 2012, Glaser *et al.*, 2000)
PyOM-C, quantity and quality (*Manuscript II, IV* and *V*)	BPCA analysis using Gas Chromatography coupled to a flame ionization detector (GC-FID)	(Brodowski *et al.*, 2005b, Glaser *et al.*, 1998, Schneider *et al.*, 2010)
Microbial biomass (*Manuscript II* and *III*)	Chloroform-fumigation and extraction	(Vance *et al.*, 1987)
Enzyme assay	Fluorescence-based soil assays for	

(*Manuscript III*)	β-1,4 glucosidase, β-N-acetylglucosaminidase, β-cellobiohydrolase and	(Sinsabaugh *et al.*, 2005)
	Colorimetric soil assays for phenol oxidase	(Pind *et al.*, 1994, Saiya-Cork *et al.*, 2002)
DNA extraction from soils (*Manuscript III*)	Modified CTAB (hexadecyltrimethylammonium bromide) extraction with Aluminum sulfate and PEG (30% polyethylene glycol in 1.6 M NaCl) precipitation followed by cleaning using Allprep kit (Qiagen Valencia CA)	(DeAngelis *et al.*, 2011)
Polymerase chain reaction (PCR) (*Manuscript III*)	From soil DNA using universal bacterial primers 907R and 515F and Takara Bio Inc. Hot start Ex-Taq polymerase in PCR thermocycler (GeneAmp PCR system 9600, Perkin-Elmer)	
Purification and concentration of PCR product (*Manuscript III*)	Solid Phase reversible immobilization (SPRI) beads (Agencourt AMPure XP, Beckman genomics, Cat A63880)	(Deangelis *et al.*, 1995)
Pyrosequencing (*Manuscript III*)	454 GS-FLX Titanium™ at UC Berkeley DNA sequencing facility	(Ronaghi, 2001)
Sequence refinement (*Manuscript III*)	Downstream analysis in the Quantitative Insights Into Microbial Ecology (QIIME) pipeline. Sequences clustered at 97% pairwise identity using the UCLUST reference-based OTU picking method Taxonomic assignments done with the naïve Bayesian algorithm developed for the RDP classifier using the GG99 taxonomy data set as training	(Caporaso *et al.*, 2010a, Cole *et al.*, 2009, Edgar, 2010, Wang *et al.*, 2007, Werner *et al.*, 2012)

4 Results and Discussion

The focus of this results and discussion section will be on the dynamics of PyOM-C and wood-C. In the following subsections, results are specifically highlighted to discuss PyOM turnover time in terrestrial system (4.1), the pathways of ^{13}C and ^{15}N of PyOM and wood (4.2, 4.3, 4.4, 4.5) and effect of PyOM and wood addition to soils on microbial activities and community structure (4.6, 4.7, 4.8). The effect of nitrogen treatment on PyOM-C dynamics (4.9) and the results of ^{13}C-BPCA-measurement technique (4.10) are detailed in subsequent sections.

4.1 PyOM turnover time in terrestrial system is on centennial scale

From evaluating studies on PyOM degradation with one consistent approach (*Manuscript I*), the turnover times of PyOM in the terrestrial system ranged from decades to hundreds of years. The scatter in the turnover time was partly explained by differences in experimental studies. The turnover time computed using one-pool decay model yielded an average value of 88 y while using model fit by non-linear regression and chi square minimization was 291 y. The two-pool decay model estimated the turnover time to be 3 y for the fast-cycling pool and 870 y for slow-cycling pool and gave a slightly better fit than the one-pool decay model. The turnover time computed varied with duration of experiment, initial biomass, pyrolysis temperature and type of medium (Fig.7).

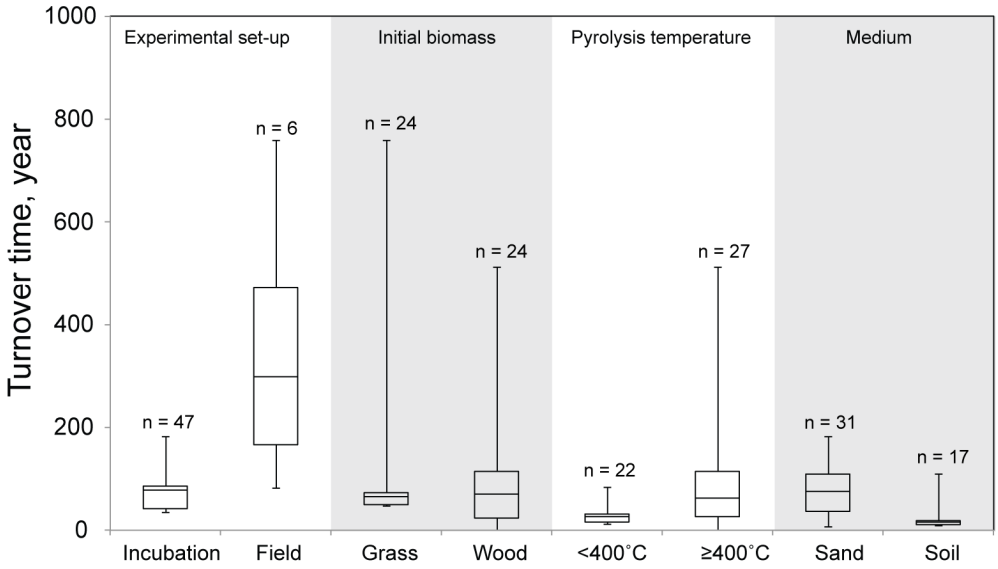

Figure 7: *Box-plot for turnover time based on different grouped factors, namely duration of experiment, initial biomass, pyrolysis temperature and medium. The n in the figure indicates number of studies used for the box plot.*

However, other factors that could influence turnover time of PyOM, for instance, climate, soil types were not estimated due to lack of sufficient scientific data. A limitation on the turnover time results is that at present, most of the degradation studies were carried under controlled conditions and for short duration. Moreover, estimating precise turnover time of PyOM is challenged due to lack in the understanding of the mechanisms pivotal for the degradation or persistence in the soil system. Nevertheless, the nominal estimate of turnover of PyOM was shorter than previously assumed and therefore requires prudence and further research to use it as a strategy for offsetting carbon emissions.

4.2 The loss of PyOM-C was negligible after 10 months

The experimental design in this thesis allowed tracking the pathway of ^{13}C labeled wood and PyOM in the soil profile. After 10 months, all of ^{13}C PyOM was recovered while only half of applied ^{13}C wood was recovered (Fig. 8). Similar trend was observed for ^{15}N recoveries for both PyOM and wood. As discussed in *Manuscript II*, the observed recoveries confirmed that PyOM decomposed slower than its initial biomass (Baldock & Smernik, 2002, Hilscher & Knicker, 2011b, Santos *et al.*, 2012).

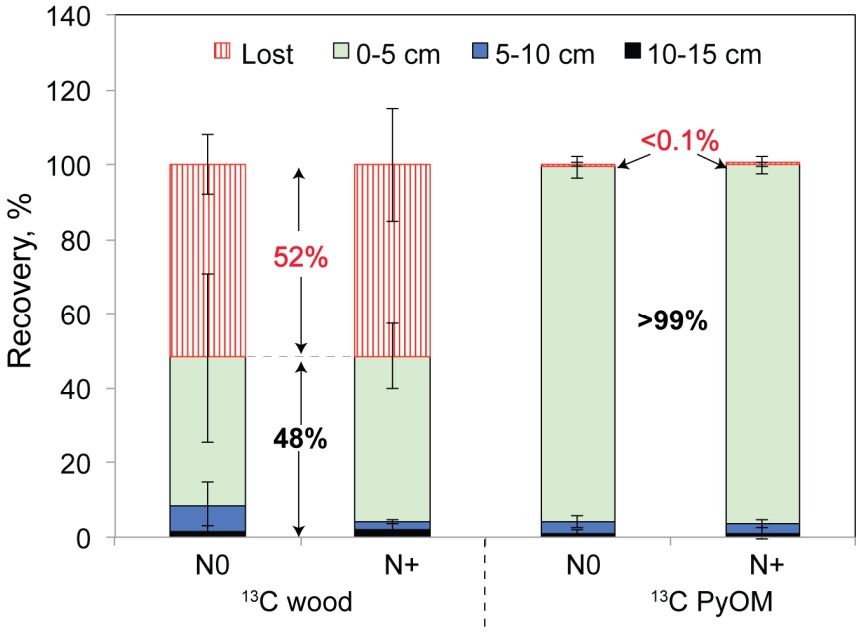

Figure 8: *The recovery of ^{13}C wood and ^{13}C PyOM after 10 months, in situ under ambient N (N0) and increased N (N+) treatment.*

The vertical movement of C and N of both PyOM and wood were limited as observed by their recoveries from depths lower than the application depth (below 2 cm from

the surface). This suggests that both wood C and PyOM C were mostly lost as CO_2. Leaching of wood C as dissolved organic carbon (DOC) from surface to the deeper soil profile is an important transport mechanism (Yano *et al.*, 2005, Zalamea *et al.*, 2007). The restricted vertical transport of wood C could be explained by either the soil texture in this field study, which is 30% clay or it could get biodegraded before leaching down to lower depth (*Manuscript II*). On the other hand, solubilization of fresh PyOM was observed to be very low (<0.3% of total PyOM-C) in a batch experiment (*Manuscript V*), which explains its lower migration rate via leaching to deeper soil profiles within a year. However, the solubility of PyOM increased strongly upon ageing and suggested that the vertical transport of PyOM to deeper soil would increase in the long term and can be considered as an important loss mechanism.

4.3 The molecular marker signature of PyOM changed within a year

Degradation of PyOM in the soil has been reported in several studies by abiotic and/or biotic oxidation (Cheng *et al.*, 2006, Zimmerman, 2010). Spectroscopic methods have revealed increase in functionalization of surfaces in PyOM macro pieces (Lehmann *et al.*, 2005, Nguyen *et al.*, 2008). Such chemical changes could alter the quality of PyOM and eventually its dynamics in the soil on a long-term. Within the first year of labeled PyOM in the soil, the total BPCA-C content of PyOM did not change. However, the BPCA pattern changed significantly for labeled PyOM used in the field study after 10 months with an increase in the benzene tetra-carboxylic acids (B4CA) and a decrease in the benzene hexa-carboxylic acids (B6CA) (Fig. 3 of *Manuscript II*). Moreover, 10 y aged PyOM used in the batch experiment also showed changes in BPCA pattern with slightly higher amount of benzene tri-carboxylic acids (B3CA) compared to fresh PyOM (*Manuscript V*). This was similar to an incubation study on PyOM decomposition by Brodowski (2005). As discussed in *Manuscript II*, other *in situ* studies either observed no change (Schneider *et al.*, 2011) or an increase in B6CA (Hammes *et al.*, 2008) after 100 y. Based on solid state ^{13}C NMR spectroscopy on degraded PyOM, Hilscher and Knicker (2011a) observed significant decrease in total-aryl groups after 28 months. This indicates a lower contribution in the formation of B6CA. It could be postulated that within this study PyOM degraded in the soil by breaking of condensed aromatic structure into smaller clusters.

4.4 Distribution of PyOM C and wood C in physically separated density fractions

After 10 months, 74% ± 4% of the added PyOM was recovered in the free light fraction (fLF) (<1.6 g cm^{-3}) while 20% ± 4% in the occluded light fraction (oLF) and 6% ± 1% in the dense fraction (DF) of the soil. Wood-derived C was mainly

recovered in the oLF (33% ± 16%) or the DF (27% ± 10%), however the distribution of wood C was highly variable within replicates. This study highlighted for the first time that significant interaction of PyOM and mineral phase of soil occurred within a year (*Manuscript II*). Previous reported studies on the interaction of PyOM with the mineral soil also reported significant interactions (Brodowski *et al.*, 2006, Glaser *et al.*, 2000, Laird *et al.*, 2008, Liang *et al.*, 2008, Vasilyeva *et al.*, 2011) but these studies do not report the temporal scale at which aggregation (oLF) and/or organo-mineral interaction (DF) of PyOM with soil occurs. The interaction of PyOM and soil mineral phase might stabilize PyOM by aggregation and organo-mineral associations (2000). As one mechanism, PyOM when gets oxidized has been hypothesized to favor its interaction with the soil mineral phase (Brodowski *et al.*, 2006). It was, however, not possible to directly link oxidized form of PyOM to its interactions with soil minerals that led to recovery of PyOM in the DF.

4.5 PyOM accelerated loss of the native soil C stock in the free light fractions

Our understanding on the occurrence, magnitude and mechanism of the interdependent decomposition of different organic input to the soil and SOM is small. One of the major effects of added organic input on the SOM is modification of its mineralization rate. Within this thesis, the effect of addition of PyOM and wood on the dynamics of native SOM was observed. The priming effect on the SOM by addition of wood or PyOM in this thesis was quantified as cumulative priming based on loss or increase in the native stock of SOC after 10 months (*Manuscript II*), Fig.9.

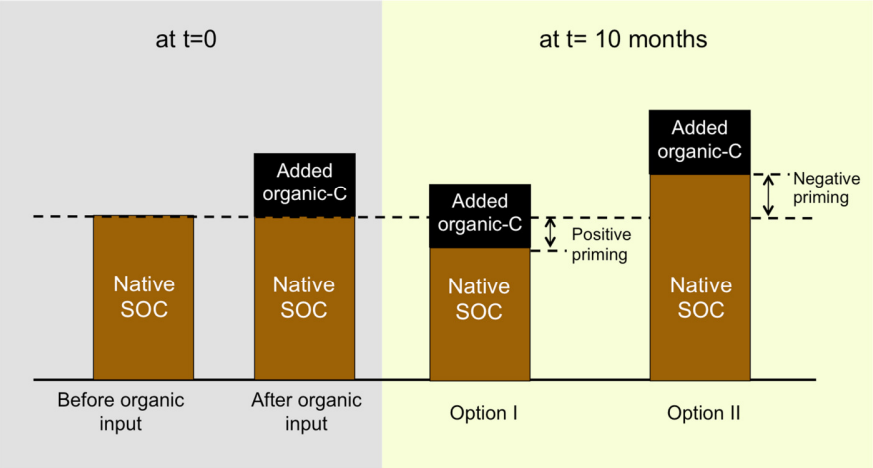

Figure 9: *The concept of cumulative priming effect (PE) on the native SOC stock due to added organic input. The cumulative PE in organic carbon amended soil was calculated as the difference in native SOC stock from unamended-control soils. This was achieved by using two end-member linear mixing model, which is based on the difference in stable isotope signature of added substrate and native SOC.*

For the first time, PE of added substrate on the native SOC stock was calculated in the physically separated soil C pool using density fractionation and stable isotope

approach. Most of the PE occurred in the fast cycling SOC pool (fLF). The native C in fLF decreased significantly for PyOM-amended soil (13% ± 1.2%). However, PE in the wood amended soil (5% ± 1.8%) was not significant. There is no consistent effect of PyOM on the native SOC across the literature. This study, for the first time, indicated the SOC pool that gets affected due to priming by organic input such as PyOM or wood. The native SOC associated to the minerals (DF) is not affected by the input while the SOC that is free (fLF) or occluded in aggregate (oLF) decreased strongly within few months.

4.6 Phenol oxidase activity increased in PyOM-amended soil

The activity for soil enzymes β-1, 4 glucosidase (BG), β-N-acetylglucosaminidase (NAH), β-cellobiohydrolase (CBH) and phenol oxidase was studied to determine if soil amended with wood or PyOM showed any difference in the soil microbial activity when compared to unamended control soils. After ten months, there was no difference observed in the enzyme activity of glucose (BG), cellulose (CBH) and chitin (NAH) digesting enzymes in the wood- or PyOM-amended soil as compared to the control-unamended soil. However, phenol oxidase activity showed significantly higher activity in the PyOM-amended soil under N0 levels. The result of this study also highlighted that added N reduced the activity of phenol oxidase in PyOM amended soil as well as BG and NAG in control soils. Notably, for the wood-amended soil, the activities of all enzymes studied here tend to be higher under added N but it was not significant. This study showed that after 10 months of application of either wood or PyOM resulted in no change in the enzyme activity, except phenol oxidase.

4.7 Microbial community structure changed at family rank in the PyOM amended soil

The soil in the present study was on average dominated by phyla *Proteobacteria* (40%, with class *Alphaproteobacteria* = 22%, *Betaproteobacteria* = 8%, *Gammaproteobacteria* = 6% and *Deltaproteobacteria* = 4%), *Actinobacteria* (28%), *Acidobacteria* (9%), *Plantomycetes* (6%), *Bacteriodete* (5%), *Gemmatimonadetes* (2%), *Firmicutes* (0.9%), *Chloroflexi* (0.06%), *Verrucomicrobia* (0.2%), and *WS3* (0.2%). At the phylum level, there was no significant difference in the relative abundance of bacteria either due to organic input or nitrogen treatment compared to the unamended-control soils. However, at finer taxonomic scale soil bacterial community composition showed significant difference across treatments. Specifically, PyOM-amended soil showed an increase in the relative abundance of family *Holophagaceae* (*Acidobacteria*), *Dermacoccaceae* and *Micrococcaceae* (*Actinobacteria*) under ambient N while *Sphingobacteriaceae* (*Bacteriodetes*) under added N. Wood-amended soil under added N treatments also resulted in increase in the members of bacteria in family *Kineosporiaceae* and *Streptosporangiaceae*

(*Actinobacteria*) and *Alcaligenaceae* (*Betaproteobacteria*) while decrease in the relative abundance of family *Microbacteriaceae* (*Actinobacteria*) under ambient N. Moreover, relative abundance of bacterial group at phylum rank responded strongly to various edaphic factors, for instance pH, C and N content of the soil.

4.8 PyOM-C was incorporated in the microbial biomass

After 10 months, PyOM and wood-amended soil had no effect on the soil microbial biomass (SMB). This is in contradiction to several studies that observed an effect on microbial biomass within hours to week time scale (Bruun *et al.*, 2011, Kolb *et al.*, 2009, Steiner *et al.*, 2008; Steinbeiss et al. 2009). However, the present study was comparatively longer than the studies cited above (10 months versus a couple of weeks) and therefore we cannot exclude that the microbial biomass could have increased in the first few weeks after PyOM addition and reverted to its initial value with course of time. Nevertheless, a small amount of PyOM-C (between 0.14–0.18 % PyOM-^{13}C) was observed within the microbial biomass 10 months after organic inputs to the soil that was two levels of magnitude smaller than ^{13}C-wood (6–10%). This study together with previous studies highlighted that the amount of PyOM incorporated into the SMB was large enough to be detected clearly, and indicated that microbes are involved and could utilize PyOM as C sources.

4.9 Added nitrogen had no effect on PyOM-C dynamics

Added N treatment had limited impact on the different parameters considered in this thesis and affected especially the wood-amended soil. There was significant increase in the total C content at 0-5 cm depth in the wood-amended soil, higher loss of wood-N and a higher transfer of wood derived C in the DF. It could be hypothesized that increased N fertilization could cause a shift in microbial community structure, towards those that could utilize N in organic substrate amended soil. In this respect, using pyrosequencing method we observed that bacterial community structure shifted with added N across all organic treatment including control-unamended soil. These data suggested a major increase in the microbes that could utilize organic substrates but were limited due to N availability. Moreover, added N had an important effect on microbes involved in nitrogen cycles (for e.g. N_2 Fixers).

4.10 Ion exchange chromatography–isotope-ratio mass spectrometry (IEC-IRMS) proved to be a suitable method for stable isotope analysis of BPCAs

This thesis presents an IEC-IRMS method for the measurement of δ^{13}C-BPCA following hydroxide gradient separation of individual BPCAs by anion-exchange

chromatography, taking advantage of the ionic properties of benzene polycarboxylic acids (*Manuscript IV*). The accuracy and precision of this method were evaluated on: (i) PyOM from both C3- and C4-plants, (ii) PyOM with and without a ^{13}C-tracer (natural abundance and artificially enriched), (iii) Mollisol soil from a C3-dominated ecosystem, (iv) artificial mixtures of C4-PyOM and C3-Mollisol soils, and (v) Spodosol soil from a C3-dominated ecosystem, representing a range of sample matrices and isotopic composition. The results showed that 30–100 mM sodium hydroxide gradient was able to sufficiently separate all B3CA through B6CA, except 1,2,4-B3CA. Further developments in sample purification, especially the removal of non-BPCA carbon, will be critical to simplifying the separation of all B3CAs. The quantification of BPCAs as measured by IEC-IRMS in Mollisol soil was ≥1:1 and slightly higher for Spodosol soil as those measured by GC-FID. The underlying mechanism for the observed differences in quantification between soil types was unknown. Further, precision estimates of the $\delta^{13}C$-BPCA values by IEC-IRMS compare favorably with those by GC-C-IRMS and the reproducibility of the $\delta^{13}C$ measurement of individual BPCAs by IEC-IRMS was better than 0.35‰(1σ). Analysis of samples that differed in $\delta^{13}C$ by as much as 900‰ revealed carryover of <1‰ between samples. Notably, the weighted sum of the IEC-IRMS measurement of individual BPCAs approximated the bulk elemental analyzer-IRMS measurements of the standard reference PyOM materials (within 5%), both at natural abundance and when artificially enriched in ^{13}C. IEC-IRMS was therefore shown as a suitable method for a range of sample types, at both natural and artificial ^{13}C-abundance. Future method improvements and its potential application for other environmental matrices are detailed in *Manuscript IV*.

5 Conclusions

A graphical conclusion of this thesis work is shown in Fig. 10. The results of this thesis contributed to the novel information to our present knowledge on the decomposition of PyOM and mechanisms responsible for its stabilization. Following were the major conclusions:

1) The turnover of PyOM-C in the soil occurs on a centennial time scale.
Based on the analysis of published data, the nominal turnover time of PyOM C is shorter than previously assumed. The computed turnover time varied between different studies (<1 y to 750 y) and was shown to depend on initial biomass type, pyrolysis temperature, and incubation or field study. Over a range of PyOM C properties and edaphic conditions, PyOM C was found to degrade in the soil, and does not act as universally recalcitrant compound in the soil. A limitation on the turnover time results was that at present, most controlled studies of PyOM C were relatively short-term and might be biased towards rapid turnover times.

2) PyOM decomposed much slower than plant biomass from which it was derived after pyrolysis, *in situ*
The mean difference of added PyOM-C and wood C stock after 10 months provided *in situ* confirmation of relative rates of decomposition reported by previous laboratory studies. After 10 months, the loss of PyOM-derived C was <1% while wood derived C was 52% of the total substrate-C.

3) PyOM shows slow downward transport in the soil profile that could increase with ageing
The field study revealed that both PyOM-C and wood-C migrated downward with course of time (10 months) and were recovered until the depth 10-15 cm indicating migration rate of 126 mm yr^{-1}. The amount of PyOM-C recovered below the application depth (0-5 cm) (3-4% of PyOM -C) was half of wood-C (4-8% of wood-C). The slow vertical movement of PyOM could be due to its partial solubility as observed in a batch experiment but it tends to increase with the ageing of PyOM in the soil.

3) About one-third of the applied PyOM C was stabilized within aggregates (i.e. oLF) plus dense fraction of soil within one year
Despite PyOM-C was mostly recovered as the free light fraction (70% of total PyOM-C), about 30% of PyOM C was incorporated within the physically protected fractions of soil (i.e. oLF and DF) in 10 months. Therefore, PyOM persistence in the soil could also be due to its protection within aggregates or formation of organo-mineral interactions.

4) PyOM C accelerated the loss of native soil C

PyOM resulted in priming native SOC, particularly in the free light fraction. We observed loss of 12% of total native SOC from the fLF. No such effect was observed for the wood-amended soil. However, further research is needed for predictive understanding and the temporal course of priming-type effects induced due to PyOM amendment of the soil.

5) PyOM breaks down into smaller aromatic clusters with time during the degradation in the soil

The quantitative measure of PyOM-C, measured as BPCA-C yield, did not change after 10 months. However, the relative contribution of the individual molecular marker changed within 10 months. Notably, aromatic condensation, measured as the relative contribution of B6CA to the total BPCA-C, of PyOM in the soil decreased after 10 months, suggesting that PyOM partially degraded into smaller aromatic moieties.

6) Added N treatment had limited effect on the decomposition dynamics of PyOM

In this field study PyOM was less sensitive to added N treatment. However wood amended soil was marginally affected with increase in wood C stock due to stabilization of wood C in protected soil fractions while higher losses of wood-N.

7) Microbial community was affected by addition of PyOM to soil at a finer taxonomic scale (e.g. at family rank)

No change in the microbial biomass C was observed in PyOM or wood amended soil. Phenol oxidase activity increased in the PyOM-amended soil while no change was observed for other enzymes studied in this thesis. PyOM-amended soil resulted in an increase in the relative abundance of bacteria within the family of phylum *Actinobacteria* and *Bacteriodetes* while decrease in the family of class *Deltaproteobacteria*. Change in the microbial community structure was also observed for the wood-amended soil under added N treatments with significant change in the relative abundance of families of phylum *Actinobacteria* and *Betaproteobacteria*. Pyrosequensing served as an effective tool to decipher specific changes in the microbial community at a fine taxonomic scale.

8) IEC-IRMS method could be a suitable alternative to measure δ^{13}C-BPCA

The IEC-IRMS approach significantly improves both the reliability and the accuracy of isotopic BPCA determinations by eliminating the complications related to derivatization using the conventional GC-C- IRMS approach. IEC-IRMS proved to be a suitable method for a range of sample types from PyOM to soils, at both natural and artificial ^{13}C-abundance.

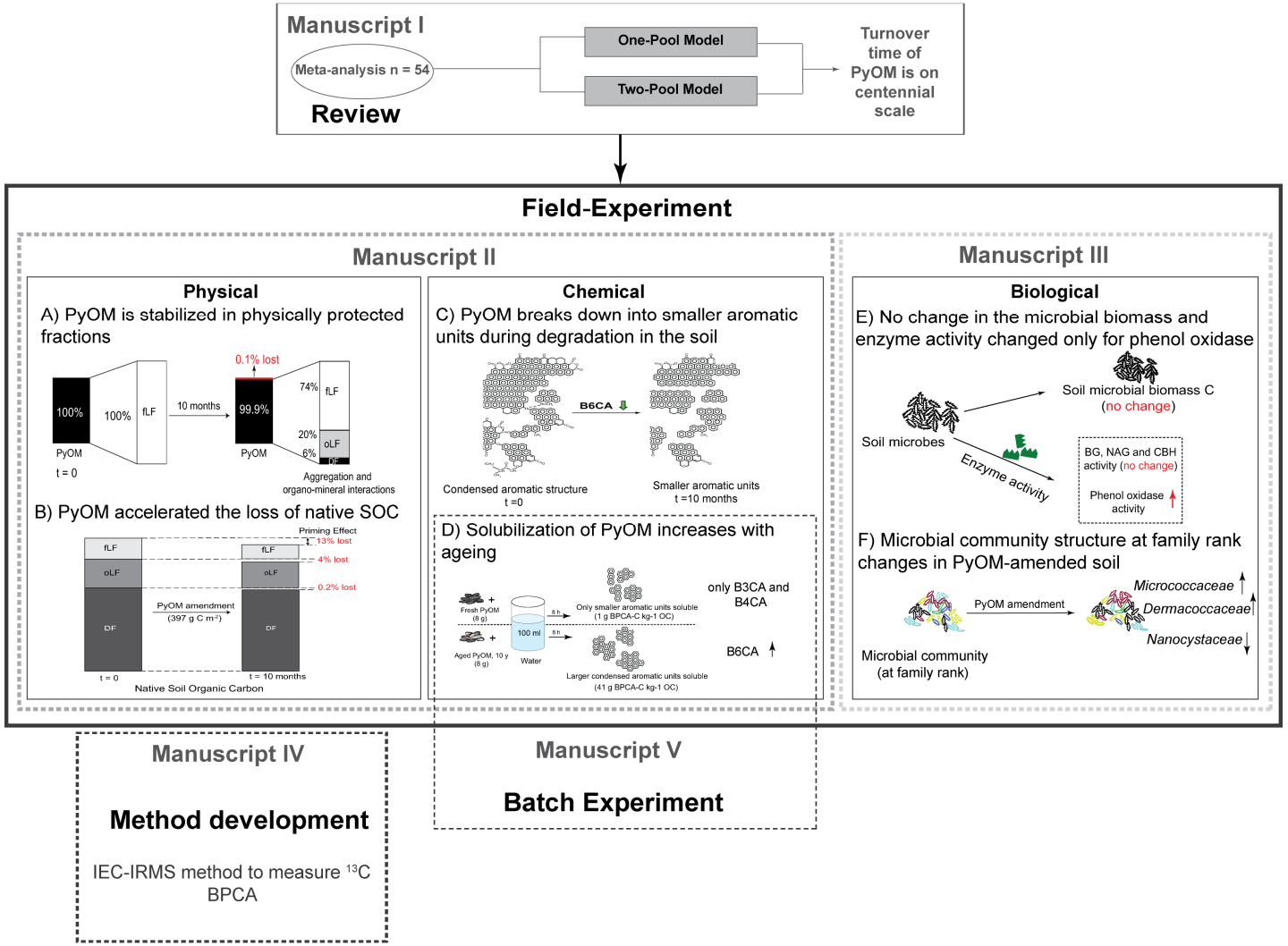

***Figure 10**: Schematic overview of the elements of this thesis and major conclusions and the grouping of various topics into manuscripts.*

6 Perspective

The understanding of the dynamics of PyOM in soil is still in its infancy and the complexity of various interdependent mechanisms poses major obstacles. Also, in the changing climate these mechanisms would alter and requires further attention. Based on the current state of research and the finding of this thesis, research topics that could be addressed in future research are outlined in the following sections.

6.1 Effect of global warming on PyOM decomposition and turnover rates

Global climate is changing and therefore it is important to focus on studies that could underline these variations in processes with change in biotic or abiotic factors. With increase in CO_2 and other greenhouse gases, future predictions highlight increase in global temperature. Experimental studies overwhelmingly indicate increased SOC decomposition at higher temperatures (Jenkinson & Ayanaba, 1977, Knorr *et al.*, 2005b, Trumbore *et al.*, 1996), resulting in increased CO_2 emissions from soils. Similar increase in the decomposition rates for PyOM could be expected with increasing temperature (Cheng & Lehmann, 2009, Cheng *et al.*, 2006). But we do not know how and on what timescale will it respond to such changes? Future efforts in the understanding of PyOM dynamics could focus on environmental variables for instance, temperature and moisture, that are important determinants for turnover time and are also subjected to change in future.

6.2 Ageing of PyOM

The change in the chemistry, molecular characteristics and solubility in water of PyOM with time has been observed in this thesis. Previous studies observed changes in physicochemical properties of PyOM with time, such as elemental composition due to oxidation (Cheng *et al.*, 2006), surface chemistry and adsorption characteristics (Cheng & Lehmann, 2009) and hydrophilicity (Chughtai *et al.*, 1991). It is likely that changes in the physical and chemical properties with time (ageing) could affect PyOM biogeochemical properties. However, there is no clear indication if PyOM becomes more labile or more stable with the observed changes. Research approaches are needed to identify changes due to ageing which are critical for its environmental significance and combine them to better elucidate PyOM storage or loss from terrestrial system. This could be done by controlled manipulative ageing experiments using chemical and biological oxidants. This is vital in filling gaps in the understanding of the decomposition dynamics of PyOM at later stages in a short observational window of a common research timeframe (<5 y).

Part B – Manuscripts

Manuscript I

Fire-derived organic carbon in soil turns over on a centennial scale

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Biogeosciences

Fire-derived organic carbon in soil turns over on a centennial scale

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Abstract. Pyrogenic carbon (PyC), the residue of an incomplete combustion of biomass, is considered as a carbon (C) sink due to its assumed stability in soil. PyC turnover time estimated using two modelling approaches, based on data from 16 published studies ($n = 54$) on PyC degradation, ranged from a decadal to centennial time scale, varying with initial biomass type, pyrolysis temperature, and incubation or field study. The average turnover time using a one-pool approach was 88 y, and the best estimate using a two-pool approach was 3 y for a fast-cycling pool and 870 y for a slow-cycling pool. Based on this meta-analysis, PyC cannot be assumed to persist in soils for thousands of years, and its use as a strategy for offsetting carbon emissions requires prudence and further research.

1 Introduction

Wildfires transfer approximately 0.05 to 0.2 Pg C yr^{-1} to soil (Seiler and Crutzen, 1980; Kuhlbusch, 1998) as incomplete combustion residue of biomass, known as pyrogenic carbon (PyC) (Goldberg, 1985). Climate change is projected to increase wildfire frequency in many parts of the world (Flannigan et al., 2006), which could modify the input of PyC and consequently the terrestrial carbon cycle (Westerling et al., 2006). PyC is ubiquitous in the environment and ranges from 2 % to 45 % of the total soil organic carbon (SOC) in terrestrial systems (Bird et al., 1999; Schmidt et al., 1999; Skjemstad et al., 2002; Lehmann et al., 2008). Some researchers suggest that PyC forms a slow-cycling C pool in the soil (Preston and Schmidt, 2006; Marschner, 2008). If so, conversion of plant biomass to PyC would represent a transfer of faster-cycling biomass-C to slower-cycling C in soils (Ohlson et al., 2009) and is therefore expected to act as a C sink (Seifritz, 1993; Marris, 2006). In the last decade, PyC has gained interest as a strategy for sequestering atmospheric CO_2 to partly offset carbon emissions (Lehmann et al., 2006).

The ability of PyC to act as a carbon sink depends on its persistence in the soil. PyC is widely considered to be relatively "inert" (Forbes et al., 2006) because PyC has been preserved in geological samples or strata (Forbes et al., 2006), archaeological sites (Schmid et al., 2002; Glaser, 2007), and old anthropogenic soils (Glaser et al., 2000; Knicker, 2011; Glaser and Birk, 2012). Moreover, in some experiments, PyC was resistant to chemical oxidants (Skjemstad et al., 1996) and contributed to the oldest soil organic carbon (SOC) pool in some Australian soils (Krull et al., 2006). Based on the ^{14}C age of PyC macro-pieces/charcoal (Pessenda et al., 2001; Schmidt et al., 2002) and budget calculations (Forbes et al., 2006), PyC age in soil has been estimated to be on the scale of hundreds to ten thousand years (Liang et al., 2008). The limitation of using radiocarbon age to estimate turnover time is that we rarely have knowledge of the input rate (or, for isolated systems, initial stock when the radiocarbon "clock" started), which would be needed to estimate turnover times.

Recent studies, however, observe transformation and mineralization of PyC over weeks to yearly timescales (Hamer et al., 2004; Bruun et al., 2008; Hilscher et al., 2009; Hilscher and Knicker, 2011) and significant losses of PyC from the soil profile in long-term field studies (Bird et al., 1999; Hammes et al., 2008b; Nguyen et al., 2008). PyC is presumed to degrade physically (Carcaillet and Talon, 1996; Carcaillet, 2001; Lehmann et al., 2003; Hammes and Schmidt, 2009) and chemically by abiotic (Lehmann et al., 2005; Cheng et al., 2006; Hockaday et al., 2006) and/or microbial agents (Potter, 1908; Shneour, 1966; Goldberg, 1985). Incubations have identified abiotic (Cheng et al., 2006) and biotic oxidation processes (Potter, 1908; Hamer et al., 2004; Kuzyakov

et al., 2009; Zimmerman, 2010) as important mechanisms of PyC degradation. Turnover times of PyC reported in most of these experimental studies ranged between a hundred and a thousand years.

These recent observations contradict the perception that PyC persists in soil for millenia. The uncertainty in PyC persistence is accompanied by a basic lack of understanding about PyC dynamics in soil. Spokas (2010) observed an increase in stability of PyC with a decrease in the O : C molar ratio of PyC. However, the correlation between half life of PyC and the O : C molar ratio was based on different methodological approaches to estimate the mean residence time of PyC. Therefore, to reconcile the apparent discrepancies between assumed persistence of PyC based on radiocarbon age and fairly rapid degradation of PyC as observed in experiments, we assembled data from published studies on PyC losses from soil and, for the first time, calculated turnover times within and across all studies with one consistent approach.

2 Materials and methods

2.1 Data set collection from the literature

We compiled data from published studies ($n = 54$ data sets from 16 studies, Supplement Table 2) on PyC degradation. We investigated turnover times of PyC using two previously published models to describe PyC decomposition and/or soil organic matter dynamics (Supplement Table 1). These models should be seen as a way of approximating characteristic time constants rather than quantifying the exact dynamics (Burnham and Anderson, 2002).

2.2 One-pool approach

In the first approach, we used a one-pool exponential decay model in which PyC is modelled as a single homogeneous C pool and assumed to follow first-order kinetics (Brodowski, 2005; Cheng et al., 2008b; Hammes et al., 2008b; Nguyen et al., 2008). We assumed that there were no new PyC inputs between time = 0 and time = t (in years). We calculated the decay rate from the total loss of PyC (sum of all loss processes including leaching, erosion, mineralization, and/or decomposition) relative to the initial stock, to estimate the turnover time of PyC in the soil with respect to all loss pathways of PyC from the soil to other terrestrial pools or from the terrestrial ecosystem.

Based on these assumptions, the decay rate k is calculated from the loss of PyC over time as follows:

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t is the remaining stock after time t, C_0 is the initial stock of PyC (at $t = 0$), and k is the decay rate (y^{-1}). The turnover time τ is calculated as $\tau = 1/k$.

For the one-pool approach, we calculated turnover times based on two data points for each study, the initial stock of PyC and final PyC remaining at the end of the experiment, using Eq. (1). Most studies had only two data points, and the intermediate points that were reported in a few studies (Hamer et al., 2004; Brodowski, 2005; Kuzyakov et al., 2009) were not included for consistency. Further, the compiled data set ($n = 54$) was used to generate a time series stock of PyC (with initial stock at time $t = 0$ being 100 % and the last point of each study corresponding to remaining stock at time t in the time series). The one-pool model was fit by constrained non-linear regression, using the chi-square minimization in the IBM SPSS statistics software package for the Mac.

2.3 Two-pool approach

In the second approach, PyC decomposition dynamics were calculated using a two-pool exponential model (Hamer et al., 2004; Hilscher et al., 2009; Kuzyakov et al., 2009; Major et al., 2009; Hilscher and Knicker, 2011).The first pool consisted of PyC with a rapid decay rate, k_{fast}, while the second pool was comprised of slowly cycling PyC and was characterized by a slow decay rate, k_{slow}. We assumed that the pools decayed in parallel – in other words that there was no exchange of PyC between pools. Thus,

$$C_t = x e^{(-k_{fast} t)} + (1 - x) e^{(-k_{slow} t)} \quad (2)$$

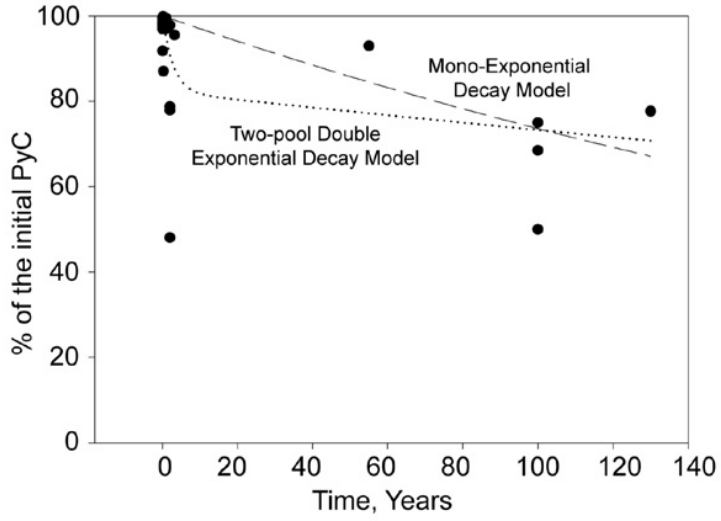
where C_t is the remaining stock after time t; x is the proportion of initial stock in the fast-cycling PyC pool (at $t = 0$), C_{fast}; $(1-x)$ is the proportion of the slow-cycling pool (at $t = 0$), C_{slow}; k_{fast} and k_{slow} are decay rate constants ($year^{-1}$).

Accordingly, the turnover time for the fast-cycling pool τ_{fast} (y) is $1/k_{fast}$ and for the slow-cycling pool τ_{slow} (y) is $1/k_{slow}$.

The two-pool model was fitted to the compiled data set ($n = 54$) (with the initial stock at time $t = 0$ and stock at the last point for each study corresponding to the time series decrease in initial stock with time) using the constrained non-linear parameter estimation procedures in the IBM SPSS statistics software package for the Mac. The curve-fitting values were iterative and required initial starting values. To avoid errors due to convergence to local minima of the residual sum of squares (RSS), we adopted convergence criteria as used by Updegraff (Updegraff et al., 1995), where final parameter estimates were accepted only if equations converged to the same values given starting values up to 50 % above and below them. The explained variance for the two-pool model is given in Supplement Table 3.

2.4 Assumptions

First, for the one-pool decay model, we accepted the simplification to one homogeneous pool, because the small amount of PyC lost within the first days suggested that the



One pool Model

$C_t = C_0 e^{-kt}$

where C_t is the remaining stock after time t (y), C_0 is the initial stock of PyC (t = 0) and k is the decay rate constant (y^{-1})

$\boldsymbol{k}$ = 0.00365 y^{-1}

∑**least square differences** = 4819.8

r^2 = 0.323

Turnover time = 1/k = 291 y

Two pool Model

$C_t = xe^{-k_{fast}\,t} + (1\text{-}x)e^{-k_{slow}\,t}$

where x is the proportion of initial stock in the fast cycling PyC pool (at t = 0), C_{fast}; $(1\text{-}x)$ is the proportion of slow cycling pool (at t = 0), C_{slow}; k_{fast} and k_{slow} are decay rate constants (y^{-1})

$C_{fast} \approx 17\%$ and $C_{slow} \approx 83\%$
$\boldsymbol{k}_{fast}$ = 0.335 y^{-1} and $\boldsymbol{k}_{slow}$ = 0.0012 y^{-1}

∑ **least square differences** = 3740.7

r^2 = 0.444

Turnover time $1/k_{fast}$ = 3 y
$1/k_{slow}$ = 870 y

Fig. 1. Parameter estimates for the one-pool exponential and the two-pool exponential model.

fast-cycling pool of PyC in most studies represented only a minor part of the whole and hence the single-pool model may be adequate to capture the bulk dynamics (Derrien and Amelung, 2011). Moreover, the assumption of one pool allowed us to compute and compare turnover times of PyC despite the differences in types of PyC, experimental setup, or analytical method employed in various studies. Further, this assumption was based on "analytical homogeneity" rather than on a "compositional homogeneity", as most studies measure a fraction of the biomass combustion continuum comprised in the mid to high temperature range. Second, first-order kinetics, meaning that the rate of mass loss is a constant proportion of mass, is a simple and robust formulation that is commonly used to describe the turnover of SOM (soil organic matter)(Parton et al., 1988). Third, the assumption of no new PyC inputs is justified because the incubation studies were set up in that way, and the field experiments were chosen where inputs had been low (although quantified poorly or not at all) after the initial sample collection. Finally, although the model yields turnover based on all loss mechanisms, we use this as a proxy for PyC decay rates, because the compiled data mostly consist of incubation studies where other loss mechanisms like erosion and leaching were limited. PyC losses by leaching do exist but are small (Abiven et al., 2011). However, erosion could be an important factor in field studies with steep slopes, as shown in Rumpel et al. (2006). However, in the field studies included in this work, erosion is probably very small – for example, in the Chernozem plains (Vasilyeva et al., 2011; Hammes et al., 2008b) and the western Kenyan plateau region (Nguyen et al., 2008).

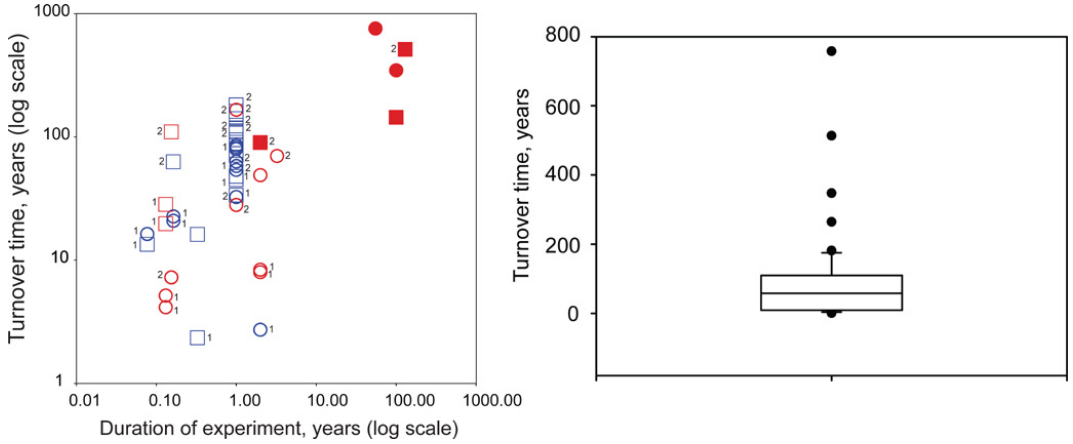


Fig. 2. PyC has an average turnover time of 88 years and ranges from <1 y to 750 y based on first-order decay model. Turnover time calculated using first-order decay vs. duration of experiment (left). The empty symbol represents incubation studies, filled symbol represents field-based studies, circles correspond to grass PyC, squares correspond to wood PyC, colour represents sand (blue) and soil (red) medium, the numbers represent temperature of pyrolysis: (1) for <400 °C and (2) for ≥400 °C. There is a weak relation between experiment duration and individual turnover time ($r^2 = 0.49$), showing experiment duration is not the only factor influencing turnover time. Box plot of individual turnover time for each study (right), where filled black circles are outliers beyond 5th or 95th percentiles.

Therefore, the data set is restricted to those studies where (1) the initial inputs and stock were known or could be estimated; (2) the initial stock decreased or remained constant with time; and (3) the experimental setup included terrestrial systems.

3 Results and discussion

3.1 Turnover time of PyC for combined dataset

The turnover time computed for each study using Eq. (1) ranged from <1 to 750 y and yielded an average value of 88 y (with standard deviation as 131 y and standard error of mean as 18). The large standard error represents a large variation in the experimental studies. The overall turnover time of PyC computed with the one-pool decay model using model fit by non-linear regression and chi square minimization was 291 y ($r^2 = 0.32$, $n = 54$, root mean square error = 10.13). The turnover time computed with the two-pool model was 3 y for the fast-cycling pool ($C_{fast} = 17\,\%$) and 870 y for the slow-cycling pool ($C_{slow} = 83\,\%$) of PyC ($r^2 = 0.44$, root mean square error = 8.35). The two-pool model gave a slightly better fit to the data than did the one-pool model (Fig. 1).

The calculated turnover times are much shorter than previously assumed or estimated to date. The higher number of short-term studies in the compiled data set, which mainly capture the fast-cycling dynamics, could influence the overall calculated turnover time to a faster value. Although having faster decay than previously thought, the overall turnover times suggest that PyC is more stable than all known plant-derived organic compound classes in soil, based on low-level ^{13}C labelling experiments (Amelung et al., 2008; Glaser, 2005).

3.2 Turnover time of PyC as a function of different factors

We observed a high scatter in the turnover times between different studies (ranged from <1 to 750 years) (Fig. 2). For instance, Brodowski (2005) observed 16–22 % PyC degradation in 104 weeks of incubation study (yielding a turnover time of 8 years), while Shindo (1991) observed no decomposition of grassland plant PyC in volcanic ash soil for 40 weeks of incubation. This scatter can be partly explained by the different experimental approaches among studies; for instance, a major difference is the experiment duration. Most PyC incubation studies lasted for a few months to a year and were potentially biased towards shorter turnover times (Derrien and Amelung, 2011).

Other factors can also be identified. Edaphic factors influence the decomposition rate of SOM (Trumbore, 2000) and could influence PyC turnover. Additionally, the types of biomass used to make PyC (Franklin, 1951), pyrolysis temperature (Schneider et al., 2010), pyrolysis conditions (e.g. inclusion or exclusion of air), and non-edaphic environmental conditions (Cheng et al., 2008a) may affect PyC turnover in soil.

We grouped the data to see whether these factors influenced turnover times when all other factors were allowed to vary, namely (1) incubation vs. field studies; (2) type of biomass (grass vs. wood); (3) pyrolysis temperature (<400 °C and ≥400 °C); and (4) quartz sand vs. soil medium (Fig. 3). Data were not grouped by other factors that control SOM decomposition – like climate, degree of

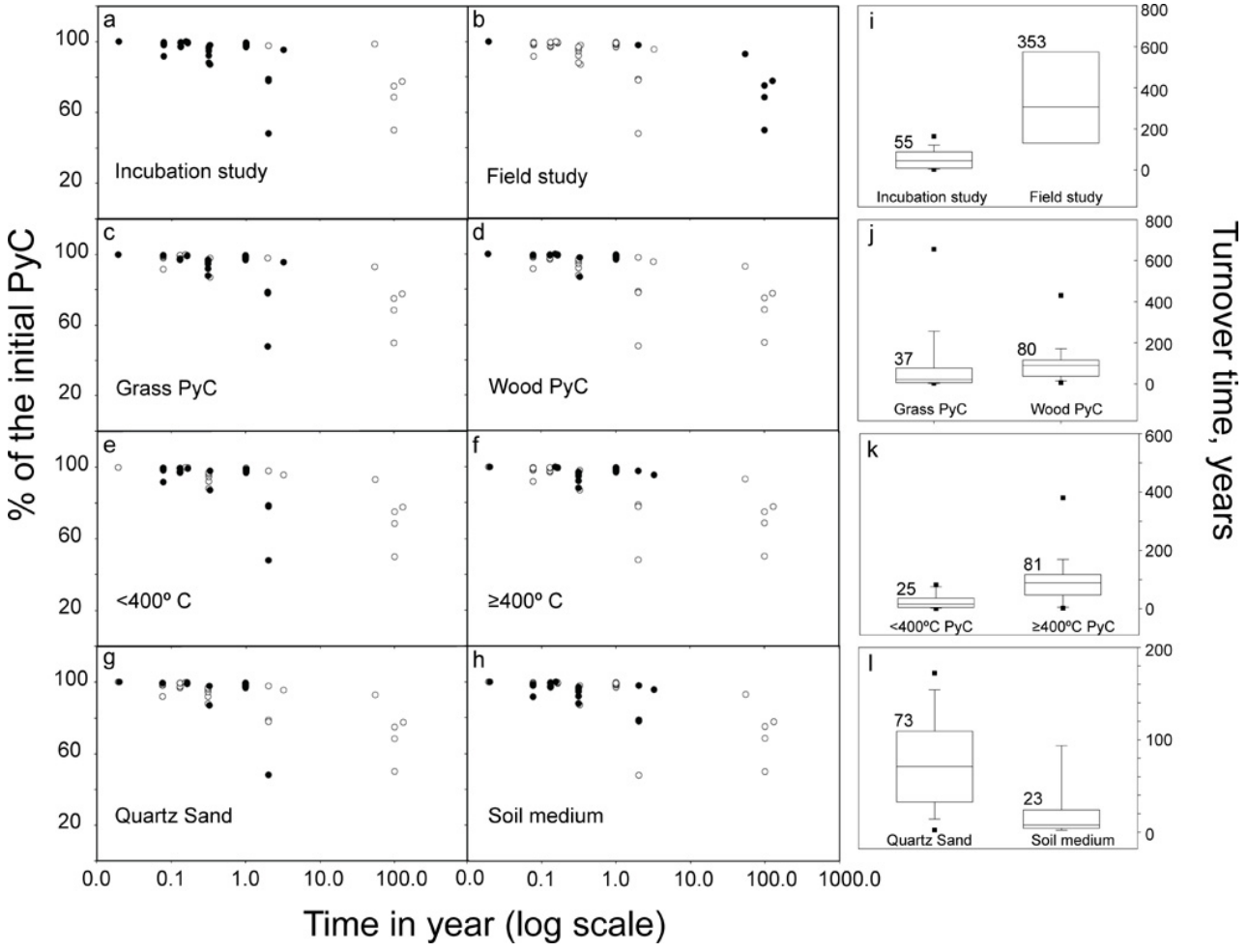


Fig. 3. Pyrogenic stock expressed as percentage of the initial from the data reported in literatures. Circles represent all data points and solid circles represent the grouped data set, namely **(a)** incubation study, **(b)** field study, **(c)** grass PyC, **(d)** wood PyC, **(e)** pyrolysis temperature <400 °C, **(f)** pyrolysis temperature ≥400 °C, **(g)** sand medium and **(h)** soil medium. The time is expressed in years (log scale). The box plot (right) of turnover time of each variable **(i)–(l)**, where squares are outliers beyond 5th or 95th percentiles and numbers denote average turnover time. The calculated turnover time varies from decades to century.

soil development, soil types, topography, and biota – because they were either not reported or were kept constant in most studies. We computed individual turnover times for each data set ($n = 54$) using Eq. (1) and computed the range, average, and variation of turnover times associated with each of the above-mentioned factors. To avoid the effect of the differences due to time scale, we only considered incubation studies and therefore used Eq. (1) for the grouped data. For comparison between incubation studies and long-term field studies, we also computed turnover time using Eq. (2) for the long-term field study to take into account the slowing down of the mineralization rate with time. The turnover times of grouped factors were compared using a non-parametric Wilcoxon rank sum test. Interactions between factors on the compiled data were evaluated by multi-way ANOVA using R software (supplementary Table 3). Our analysis shows that we do not have any significant interactions between the factors. Therefore, the unbalanced design of the grouped data does not introduce any significant error in the interpretation, and we can evaluate the differences in turnover times associated with these different factors.

3.2.1 Incubation vs. field study

Incubation studies have significantly ($p < 0.001$) shorter turnover times (average 55 y; range 1–180 y) than field-based studies (average 353 years; ranges from 90 to 750 years), computed using the one-pool decay model, Eq. (1) (Fig. 3). Short-term decay studies may primarily capture the rapid initial loss of more labile or/and non-charred components, and therefore may not be a good indication of the long-term degradation rates. The two-pool model partly solves this problem, but we had insufficient information to parameterize this model for individual studies; the two-pool model results

reported above were generated with the combined dataset. The turnover time computed on a combined data set for incubation studies ($n = 47$) using the two-pool model approach showed a low value for regression ($r^2 = 0.16$); hence it is not reported here (Supplement Fig. 1). These possible explanations for the short turnover time in incubations also pertain to short-term field experiments (Major et al., 2009). Incubation studies were conducted with fine-sized PyC (Hamer et al., 2004; Major et al., 2009; Nocentini et al., 2010b) at higher or constant room temperature and field capacity moisture content (Baldock and Smernik, 2002; Hilscher et al., 2009; Zimmerman, 2010), which partly explains the accelerated degradation rates and the faster turnover time (Nocentini et al., 2010a).

Long-term field studies provide a more realistic estimation of in situ turnover time of PyC, which not only includes the rapid initial phase but also the phase when the mineralization rate decreases with time. We took advantage of published data from long-term field studies to estimate the turnover time for PyC in situ. It was, however, not possible to conduct a straightforward mass balance for the multi-year field studies, because few of these had data on initial PyC stock and the rate of atmospheric deposition of PyC throughout each study was unknown. The turnover time derived by analysing all the data from long-term field studies ($n = 6$) using the two-pool model was 91 y for the fast-cycling pool and 1034 y for the slow-cycling pool, with 49 % in the fast pool ($r^2 = 0.51$) (Supplement Fig. 1). Thus, long-term field data indicate that a significant fraction of PyC turns over on roughly centennial scale, which is shorter than previously assumed or estimated (Lehmann et al., 2008; Liang et al., 2008; Kuzyakov et al., 2009). At present, little is known about the underlying mechanisms of PyC degradation on longer time scales. Decomposition under field conditions may be enhanced by freeze–thaw cycles (Carcaillet, 2001), root growth (Carcaillet and Talon, 1996; Lehmann et al., 2003), fungal hyphae (Hammes and Schmidt, 2009), soil fauna (Ponge et al., 2006), and erosion that exposes PyC to biological and chemical degradation.

3.2.2 Initial biomass type

There were two types of initial biomass in the studies we used that were representative for grassland and forest ecosystems, namely grass and wood (Fig. 3). Grass PyC turned over (average 37 y, range 2–170 y) significantly faster ($p < 0.05$) than wood PyC (average turnover time = 79 y, range 2–181 y) in the incubation studies. This is consistent with a previous observation of faster oxidation of grass PyC as compared to wood, possibly reflecting differences in their chemical structure (Nguyen and Lehmann, 2009). Fourier Transformed Infra Red (FTIR) spectra of grass and wood PyC produced at the same temperature (Keiluweit et al., 2010) show differences in the physical architecture and molecular composition of PyC produced. Knicker et al. (2008) proposed that a significant amount of grass-derived PyC consists of N-heteroaromatic carbon, with the average cluster size of the aromatic units smaller than six rings. In general, this would be consistent with the view that a lignocelluloses-rich substrate like wood is transformed by charring into a more aromatic structure than is the thermally labile hemicellulosic structure of grass (Czimczik et al., 2002). If true, grass PyC would probably be easier to degrade as compared to wood PyC. The influence of initial biomass on chemical structure diminishes with increasing pyrolysis temperature of PyC (Schneider et al., 2011). Therefore, the chemical and physical structure of PyC is not directly correlated with or predicable by the structure of the plant substrate for charring.

3.2.3 Pyrolysis temperature

The pyrolysis condition under which PyC is formed also determines its chemical and physical properties and possibly its turnover times. In natural environments, it is unlikely that any one set of formation conditions can be viewed as typical (Brown et al., 2006) and PyC formed during wildfire varies significantly depending on formation conditions (Schmidt and Noack, 2000). In recent years, considerable effort has been expended to characterize PyC produced naturally (Kuhlbusch, 1995; Otto et al., 2006; Smernik et al., 2006; Kaal et al., 2008; Lehmann et al., 2005) and under controlled conditions (Shindo, 1991; Pastorova et al., 1993, 1994; Nishimiya et al., 1998; Hammes et al., 2008a; Cheng and Lehmann, 2009; Keiluweit et al., 2010; Schneider et al., 2010; Zimmerman, 2010). These studies often describe PyC as a continuum from partially charred plant materials to charcoal to soot (Preston and Schmidt, 2006); however, the basic structure of PyC remains similar and consists of condensed aromatic clusters. It has been observed that high temperatures of pyrolysis and thermal ramping rates have major effects on char properties (Mackay and Roberts, 1982; Byrne, 1996; Lewis, 1999; Kercher and Nagle, 2003) and degree of condensation (McBeath and Smernik, 2009; Schneider et al., 2010). Therefore, in this review we analyse the effect of PyC produced at lower temperature and higher temperature on turnover times, rather than the effect of wildfire PyC and laboratory-produced PyC on turnover times.

We chose 400 °C as temperature threshold based on PyC thermosequence studies (Keiluweit et al., 2010; Schneider et al., 2010), which showed maximum modification in PyC structure around 400 °C. Pyrolysis temperature data were only available for incubation-based studies. Turnover time was significantly shorter ($p < 0.05$) for low temperature, <400 °C (average turnover time = 25 y, range 2–82 y), than PyC formed at high temperature, ≥400 °C (average turnover time = 81 y, range 2–181 y). Lower-temperature PyC may contain more uncharred material in the initial biomass (Zimmerman, 2010), which may be comparatively more labile than charred biomass and thus results in a faster turnover time. Moreover, PyC formed at lower temperature can have

greater internal microporosity (Hammes et al., 2008a), allowing easier access to oxidizing agents like water or microbes that facilitate degradation. The degree of condensation increases with higher pyrolysis temperature (Nishimiya et al., 1998; Baldock and Smernik, 2002; McBeath and Smernik, 2009; Schneider et al., 2010) and this could explain the slower turnover of high-temperature PyC.

3.2.4 Quartz sand and soil medium

Most incubation studies used either quartz sand with microbial inoculum or fresh soil, and all field studies took place in soil. As a consequence, we only used incubation studies to compare turnover times by medium. PyC has a shorter turnover time in soil (average = 23 y, range 2–109 y) than in quartz sand (average = 73 y, range 2–181 y). Quartz sand probably has a higher permeability and oxygen level than soil (Zimmerman, 2010), whereas soil as a medium should yield more realistic values because (among other reasons) it holds a larger range of microbial populations than that afforded by a microbial inoculum (Riis et al., 1998). The faster turnover time in soil may reflect the role of microbial community in PyC degradation. However, the influence of microbial community composition on PyC degradation is poorly understood (Pietikainen et al., 2000; Czimczik and Masiello, 2007). In addition, soils contain non-pyrogenic organic matter that may act as primer for the faster degradation of PyC (Hamer et al., 2004).

PyC is also known to interact with soil minerals (Piccolo et al., 1997; Glaser et al., 2000; Brodowski et al., 2005; Liang et al., 2008), in some cases resulting in aggregation (Brodowski et al., 2006; Vasilyeva et al., 2011) and stabilization in the soil system. However, short-term incubation studies might not capture the stabilizing effects of organo-mineral interactions.

3.2.5 Climate

Climate, including temperature and moisture, influences SOM and PyC decomposition, but there were insufficient data with which we could analyse its effect on turnover time. A few studies observed a positive correlation between mean annual temperature of the field site and PyC degradation (Glaser and Amelung, 2003; Cheng et al., 2008a) but not with mean annual precipitation (Cheng et al., 2008a). Further, Nguyen et al. (2010) also showed that degradation rates of PyC are accelerated with increasing temperature. Faster turnover of $<$100 years observed in tropical (Nguyen et al., 2008) and subtropical climate (Bird et al., 1999) was attributed to the more favourable climate. Slower degradation rates could be expected in boreal forests (Preston and Schmidt, 2006). However, in a boreal forest (Ohlson et al., 2009) PyC content decreased to the concentration of the surrounding organic soil matrix in about 100 years. Thus, we need to directly examine a range of climatic conditions to understand the influence of environment on PyC turnover time.

3.3 Discussion

Some observations of PyC content or radiocarbon age in soil have interpreted PyC as recalcitrant with turnover times in soil of millennia. Applying a simple modelling approach to a broad set of published data, we find that turnover of PyC in soil occurs faster, on a centennial time scale. This is in accordance with Hammes et al. (2008b), Major et al. (2009) and Kuzyakov et al. (2009), but significantly shorter than the turnover time estimated in an incubation study (Zimmerman, 2010) or inferred from the radiocarbon age of PyC in some studies (Pessenda et al., 2001; Schmidt et al., 2002; Liang et al., 2008). There are two considerations that help reconcile the apparent inconsistency.

First, the apparent inconsistency of PyC radiocarbon ages with the estimated turnover time of PyC could be explained by the "inbuilt age" of a piece of charcoal produced during fire because the wood may have been old at the time of the fire (Gavin et al., 2003). Few trees live to be a thousand years old, so it is likely that a piece of charcoal that is thousands of years old has been in the soil for $>$1000 years. Moreover, it is difficult to translate the radiocarbon age of an isolated piece of charcoal to a turnover time without knowledge of the initial stock or input of PyC, leading to uncertainty in the estimate.

Second, it is likely that some PyC stays in some soils for many thousands of years. The two-pool model shows that the PyC experiments analysed contained fairly slow-cycling material, even if the bulk behaviour was well described with a shorter turnover time. Studies of bulk soil organic matter find a spectrum of turnover times, with persistence depending on the compound chemistry and its physico-chemical state in soil, such as interaction with minerals or protection inside aggregate structures (Schmidt et al., 2011) that result in turnover times up to thousands of years. We would expect PyC to have similar behaviour in soil (Torn et al., 2002).

A caveat on our turnover time results is that at present, most controlled studies of PyC are relatively short-term and may be biased towards rapid turnover times, given the initial decomposition dynamics that diminish over time (Kuzyakov et al., 2009) as the labile component is metabolized (Smith et al., 2010). Our knowledge of the later stages of degradation is much poorer. Therefore, our estimates using a one-pool model could overestimate the rate of PyC degradation (and underestimate turnover time). The two-pool decay model provides a better fit to the data than the one-pool model, and does show a more persistent fraction of PyC. It has been suggested that physical protection and interactions with soil minerals play a significant part in long-term PyC stability (Brodowski et al., 2006; Glaser et al., 2000) and forming what we called the slow PyC pool. However, the duration

of available data is too short to quantify longer decay time scales.

The differences in turnover times among studies could also be due to the interplay of different decomposition or stabilization mechanisms at a local scale, resulting in differences in the rate of degradation. A combination of physical, chemical, and microbial processes can play a role in PyC degradation. However, these degradation processes have not yet been studied in combination, and questions remain as to the interaction of these processes and the importance of PyC chemistry, soil conditions, and microbial activity in controlling the likelihood of PyC degradation or persistence.

4 Conclusion and future research

PyC comprises an array of compounds and is present in different environmental matrices; thus, there is not a single rate of decomposition to describe PyC dynamics in all soils and conditions. Nevertheless, based on this analysis of published data, the nominal turnover time of PyC is shorter than previously assumed, on order of hundreds of years. Over a range of PyC properties and edaphic conditions, PyC was found to degrade in soil, and there was consistent evidence that PyC does not act as an inert or universally recalcitrant compound in soil. To better understand PyC as a potential dynamic link between fire, soil, and the carbon cycle, and to investigate its potential for carbon sequestration strategies, we recommend the initiation of long-term PyC degradation field experiments, in different climate and soil types, to address the decomposition dynamics of aging PyC, as well as research to identify underlying mechanisms of PyC degradation and the factors controlling its stability in soil.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/2847/2012/bg-9-2847-2012-supplement.pdf.

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References

Abiven, S., Hengartner, P., Schneider, M. P. W., Singh, N., and Schmidt, M. W. I.: Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal, Soil Biol. Biochem., 43, 1615–1617, doi:10.1016/J.Soilbio.2011.03.027, 2011.

Amelung, W., Brodowski, S., Sandhage-Hofmann, A., and Bol, R.: Combining Biomarker with Stable Isotope Analyses for Assessing the Transformation and Turnover of Soil Organic Matter, Advances in Agronomy, 100, 155–250, doi:10.1016/S0065-2113(08)00606-8, 2008.

Baldock, J. A. and Smernik, R. J.: Chemical composition and bioavailability of thermally, altered Pinus resinosa (Red Pine) wood, Organic Geochemistry, 33, 1093–1109, Pii S01466-6380(02)0062-1, 2002.

Bird, M. I., Moyo, C., Veenendaal, E. M., Lloyd, J., and Frost, P.: Stability of elemental carbon in a savanna soil, Global Biogeochemical Cycles, 13, 923–932, 1999.

Brodowski, S.: Origin, function, and reactivity of black carbon in the arable soil environment, PhD Thesis, Institut für Bodenkunde, Bonn, 183, 2005.

Brodowski, S., Amelung, W., Haumaier, L., Abetz, C., and Zech, W.: Morphological and chemical properties of black carbon in physical soil fractions as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy, Geoderma, 128, 116–129, doi:10.1016/J.Geoderma.2004.12.019, 2005.

Brodowski, S., John, B., Flessa, H., and Amelung, W.: Aggregate-occluded black carbon in soil, European Journal of Soil Science, 57, 539–546, doi:10.1111/j.1365-2389.2006.00807.x, 2006.

Brown, R. A., Kercher, A. K., Nguyen, T. H., Nagle, D. C., and Ball, W. P.: Production and characterization of synthetic wood chars for use as surrogates for natural sorbents, Organic Geochemistry, 37, 321–333, doi:10.1016/J.Orggeochem.2005.10.008, 2006.

Bruun, S., Jensen, E. S., and Jensen, L. S.: Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration, Organic Geochemistry, 39, 839–845, doi:10.1016/J.Orggeochem.2008.04.020, 2008.

Burnham, K. P. and Anderson, D. R.: Model selection and multimodel inference: a practical information-theoretic approach, Springer, New York, 488 pp., 2002.

Byrne, C.: Polymer, ceramic and carbon composites derived from wood, PhD, Johns Hopkins University, Baltimore, 1996.

Carcaillet, C.: Are Holocene wood-charcoal fragments stratified in alpine and subalpine soils? Evidence from the Alps based on AMS C-14 dates, Holocene, 11, 231–242, 2001.

Carcaillet, C. and Talon, B.: A view of the wood charcoal stratigraphy and dating in soil: A case study of some soils from the French Alps., Geographie Physique Et Quaternaire, 50, 233–244, 1996.

Cheng, C. H. and Lehmann, J.: Ageing of black carbon along a temperature gradient, Chemosphere, 75, 1021–1027, doi:10.1016/j.chemosphere.2009.01.045, 2009.

Cheng, C. H., Lehmann, J., Thies, J. E., Burton, S. D., and Engelhard, M. H.: Oxidation of black carbon by biotic and abiotic processes, Organic Geochemistry, 37, 1477–1488, doi:10.1016/j.orggeochem.2006.06.022, 2006.

Cheng, C. H., Lehmann, J., and Engelhard, M. H.: Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence, Geochim. Cosmochim. Ac., 72, 1598–1610, doi:10.1016/J.Gca.2008.01.010, 2008a.

Cheng, C. H., Lehmann, J., Thies, J. E., and Burton, S. D.: Stability of black carbon in soils across a climatic gradient, J. Geophys. Res.-Biogeosciences, 113, G02027, doi:10.1029/2007jg000642, 2008b.

Czimczik, C. I. and Masiello, C. A.: Controls on black carbon storage in soils, Global Biogeochem. Cycles, 21, Gb3005, doi:10.1029/2006gb002798, 2007.

Czimczik, C. I., Preston, C. M., Schmidt, M. W. I., Werner, R. A., and Schulze, E. D.: Effects of charring on mass, organic carbon, and stable carbon isotope composition of wood, Organic Geochemistry, 33, 1207–1223, Pii S0146-6380(02)00137-7, 2002.

Derrien, D. and Amelung, W.: Computing the mean residence time of soil carbon fractions using stable isotopes: impacts of the model framework, European Journal of Soil Science, 62, 237–252, doi:10.1111/j.1365-2389.2010.01333.x, 2011.

Flannigan, M., Amiro, B., Logan, K., Stocks, B., and Wotton, B.: Forest Fires and Climate Change in the 21st Century, Mitigation and Adaptation Strategies for Global Change, 11, 847–859, doi:10.1007/s11027-005-9020-7, 2006.

Forbes, M. S., Raison, R. J., and Skjemstad, J. O.: Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems, Sci. Total Environ., 370, 190–206, doi:10.1016/j.scitotenv.2006.06.007, 2006.

Franklin, R. E.: Crystallite Growth in Graphitizing and Non-Graphitizing Carbons, Proceedings of the Royal Society of London Series a-Mathematical and Physical Sciences, 209, 196–218, 1951.

Gavin, D. G., Brubaker, L. B., and Lertzman, K. P.: Holocene fire history of a coastal temperate rain forest based on soil charcoal radiocarbon dates, Ecology, 84, 186–201, 2003.

Glaser, B.: Compound-specific stable-isotope (delta C-13) analysis in soil science, J. Plant Nutr. Soil Sci.-Z. Pflanzenernahr. Bodenkd., 168, 633–648, 2005.

Glaser, B.: Prehistorically modified soils of central Amazonia: a model for sustainable agriculture in the twenty-first century, Philos. T. R. Soc. B, 362, 187–196, doi:10.1098/Rstb.2006.1978, 2007.

Glaser, B. and Amelung, W.: Pyrogenic carbon in native grassland soils along a climosequence in North America, Global Biogeochem. Cycles, 17, 1064, doi:10.1029/2002gb002019, 2003.

Glaser, B. and Birk, J. J.: State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (terra preta de Indio), Geochim. Cosmochim. Acta, 82, 39–51, doi:10.1016/J.Gca.2010.11.029, 2012.

Glaser, B., Balashov, E., Haumaier, L., Guggenberger, G., and Zech, W.: Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region, Organic Geochemistry, 31, 669-678, 2000.

Goldberg, E. D.: Black carbon in the environment: properties and distribution, John Wiley and Sons, New York, 198 pp., 1985.

Hamer, U., Marschner, B., Brodowski, S., and Amelung, W.: Interactive priming of black carbon and glucose mineralisation, Organic Geochemistry, 35, 823–830, doi:10.1016/J.Orggeochem.2004.03.003, 2004.

Hammes, K. and Schmidt, M. W. I.: Changes of biochar in soil, in: Biochar for environmental Management, edited by: Lehmann, J., and Joseph, S., Biochar for Environmental Management, Earthscan, London, 169–182, 2009.

Hammes, K., Smernik, R. J., Skjemstad, J. O., and Schmidt, M. W. I.: Characterisation and evaluation of reference materials for black carbon analysis using elemental composition, colour, BET surface area and C-13 NMR spectroscopy, Appl. Geochemistry, 23, 2113–2122, doi:10.1016/J.Apgeochem.2008.04.023, 2008a.

Hammes, K., Torn, M. S., Lapenas, A. G., and Schmidt, M. W. I.: Centennial black carbon turnover observed in a Russian steppe soil, Biogeosciences, 5, 1339–1350, doi:10.5194/bg-5-1339-2008, 2008b.

Hilscher, A. and Knicker, H.: Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment, Organic Geochemistry, 42, 42–54, doi:10.1016/J.Orggeochem.2010.10.005, 2011.

Hilscher, A., Heister, K., Siewert, C., and Knicker, H.: Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil, Organic Geochemistry, 40, 332–342, doi:10.1016/j.orggeochem.2008.12.004, 2009.

Hockaday, W. C., Grannas, A. M., Kim, S., and Hatcher, P. G.: Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil, Organic Geochemistry, 37, 501–510, doi:10.1016/J.Orggeochem.2005.11.003, 2006.

Kaal, J., Brodowski, S., Baldock, J. A., Nierop, K. G. J., and Cortizas, A. M.: Characterisation of aged black carbon using pyrolysis-GC/MS, thermally assisted hydrolysis and methylation (THM), direct and cross-polarisation C-13 nuclear magnetic resonance (DP/CP NMR) and the benzenepolycarboxylic acid (BPCA) method, Organic Geochemistry, 39, 1415–1426, doi:10.1016/J.Orggeochem.2008.06.011, 2008.

Keiluweit, M., Nico, P. S., Johnson, M. G., and Kleber, M.: Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar), Environ. Sci. Technol., 44, 1247–1253, doi:10.1021/es9031419, 2010.

Kercher, A. K. and Nagle, D. C.: Microstructural evolution during charcoal carbonization by X-ray diffraction analysis, Carbon, 41, 15–27, 2003.

Knicker, H.: Pyrogenic Organic Matter in Soil: Its Origin and Occurrence, its Chemistry and Survival in Soil Environments, Quaternary International, 243, 251–263, doi:10.1016/j.quaint.2011.02.037, 2011.

Knicker, H., Hilscher, A., Gonzalez-Vila, F. J., and Almendros, G.: A new conceptual model for the structural properties of char produced during vegetation fires, Organic Geochemistry, 39, 935–939, doi:10.1016/J.Orggeochem.2008.03.021, 2008.

Krull, E. S., Swanston, C. W., Skjemstad, J. O., and McGowan, J. A.: Importance of charcoal in determining the age and chemistry of organic carbon in surface soils, J. Geophys. Res.-Biogeosciences, 111, G04001, doi:10.1029/2006jg000194, 2006.

Kuhlbusch, T. A. J.: Methods for determining black carbon in residues of vegetation fires, Environ. Sci. Technol., 29, 2695–2702, 1995.

Kuhlbusch, T. A. J.: Black carbon and the carbon cycle, Science, 280, 1903–1904, 1998.

Kuzyakov, Y., Subbotina, I., Chen, H. Q., Bogomolova, I., and Xu, X. L.: Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling, Soil Biol. Biochem., 41, 210–219, 2009.

Lehmann, J., da Silva, J. P., Steiner, C., Nehls, T., Zech, W., and Glaser, B.: Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments, Plant and Soil, 249, 343–357, 2003.

Lehmann, J., Liang, B. Q., Solomon, D., Lerotic, M., Luizao, F., Kinyangi, J., Schafer, T., Wirick, S., and Jacobsen, C.: Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy for mapping nano-scale distribution of organic carbon forms in soil: Application to black carbon particles, Global Biogeochem. Cycles, 19, Gb1013, doi:10.1029/2004gb002435, 2005.

Lehmann, J., Gaunt, J., and Rondon, M.: Bio-Char sequestration in terrestrial ecosystems – A review, Mitigation and Adaptation Strategies for Global Change, 11, 403–427, doi:10.1007/s11027-005-9006-5, 2006.

Lehmann, J., Skjemstad, J., Sohi, S., Carter, J., Barson, M., Falloon, P., Coleman, K., Woodbury, P., and Krull, E.: Australian climate-carbon cycle feedback reduced by soil black carbon, Nature Geosci., 1, 832–835, doi:10.1038/ngeo358, 2008.

Lewis, A. C.: Production and Characterization of Structural Active Carbon from Wood Precursors, Johns Hopkins University, 192 pp., 1999.

Liang, B., Lehmann, J., Solomon, D., Sohi, S., Thies, J. E., Skjemstad, J. O., Luizao, F. J., Engelhard, M. H., Neves, E. G., and Wirick, S.: Stability of biomass-derived black carbon in soils, Geochim. Cosmochim. Ac., 72, 6069–6078, doi:10.1016/j.gca.2008.09.028, 2008.

Mackay, D. M. and Roberts, P. V.: The Dependence of Char and Carbon Yield on Lignocellulosic Precursor Composition, Carbon, 20, 87–94, 1982.

Major, J., Lehmann, J., Rondon, M., and Goodale, C.: Fate of soil-applied black carbon: downward migration, leaching and soil respiration, Glob. Change Biol., 16, 1366–1379, doi:10.1111/j.1365-2486.2009.02044.x, 2009.

Marris, E.: Putting the carbon back: Black is the new green, Nature, 442, 624–626, doi:10.1038/442624a, 2006.

Marschner, B.: How relevant is recalcitrance for the stabilization of organic matter in soils?, Journal of plant nutrition and soil science, 171, 91–110, doi:10.1002/jpln.200700049, 2008.

McBeath, A. V. and Smernik, R. J.: Variation in the degree of aromatic condensation of chars, Organic Geochemistry, 40, 1161–1168, doi:10.1016/J.Orggeochem.2009.09.006, 2009.

Nguyen, B. T. and Lehmann, J.: Black carbon decomposition under varying water regimes, Organic Geochemistry, 40, 846–853, doi:10.1016/j.orggeochem.2009.05.004, 2009.

Nguyen, B. T., Lehmann, J., Kinyangi, J., Smernik, R., Riha, S. J., and Engelhard, M. H.: Long-term black carbon dynamics in cultivated soil, Biogeochemistry, 89, 295–308, doi:10.1007/S10533-008-9220-9, 2008.

Nguyen, B. T., Lehmann, J., Hockaday, W. C., Joseph, S., and Masiello, C. A.: Temperature Sensitivity of Black Carbon Decomposition and Oxidation, Environ. Sci. Technol., 44, 3324–3331, doi:10.1021/es903016y, 2010.

Nishimiya, K., Hata, T., Imamura, Y., and Ishihara, S.: Analysis of chemical structure of wood charcoal by X-ray photoelectron spectroscopy, J. Wood Sci., 44, 56–61, 1998.

Nocentini, C., Certini, G., Knicker, H., Francioso, O., and Rumpel, C.: Nature and reactivity of charcoal produced and added to soil during wildfire are particle-size dependent, Organic Geochemistry, 41, 682–689, doi:10.1016/J.Orggeochem.2010.03.010, 2010a.

Nocentini, C., Guenet, B., Di Mattia, E., Certini, G., Bardoux, G., and Rumpel, C.: Charcoal mineralisation potential of microbial inocula from burned and unburned forest soil with and without substrate addition, Soil Biol. Biochem., 42, 1472–1478, doi:10.1016/J.Soilbio.2010.05.009, 2010b.

Ohlson, M., Dahlberg, B., Okland, T., Brown, K. J., and Halvorsen, R.: The charcoal carbon pool in boreal forest soils, Nature Geosci., 2, 692–695, doi:10.1038/Ngeo617, 2009.

Otto, A., Gondokusumo, R., and Simpson, M. J.: Characterization and quantification of biomarkers from biomass burning at a recent wildfire site in Northern Alberta, Canada, Appl. Geochemistry, 21, 166–183, doi:10.1016/J.Apgeochem.2005.09.007, 2006.

Parton, W. J., Stewart, J. W. B., and Cole, C. V.: Dynamics of C, N, P and S in Grassland Soils – a Model, Biogeochemistry, 5, 109–131, 1988.

Pastorova, I., Arisz, P. W., and Boon, J. J.: Preservation of D-Glucose-Oligosaccharides in Cellulose Chars, Carbohydrate Res., 248, 151–165, 1993.

Pastorova, I., Botto, R. E., Arisz, P. W., and Boon, J. J.: Cellulose char structure- A combined analytical PY-GC-MS FTIR, and NMR-study, Carbohydrate Res., 262, 27–47, 1994.

Pessenda, L. C. R., Gouveia, S. E. M., and Aravena, R.: Radiocarbon dating of total soil organic matter and humin fraction and its comparison with C-14 ages of fossil charcoal, Radiocarbon, 43, 595–601, 2001.

Piccolo, A., Pietramellara, G., and Mbagwu, J. S. C.: Use of humic substances as soil conditioners to increase aggregate stability, Geoderma, 75, 267–277, 1997.

Pietikainen, J., Kiikkila, O., and Fritze, H.: Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus, Oikos, 89, 231–242, 2000.

Ponge, J. F., Topoliantz, S., Ballof, S., Rossi, J. P., Lavelle, P., Betsch, J. M., and Gaucher, P.: Ingestion of charcoal by the Amazonian earthworm Pontoscolex corethrurus: A potential for tropical soil fertility, Soil Biol. Biochem., 38, 2008–2009, doi:10.1016/J.Soilbio.2005.12.024, 2006.

Potter, M. C.: Bacteria as agents in the oxidation of amorphous carbon, P R Soc Lond B-Conta, 80, 239–259, 1908.

Preston, C. M. and Schmidt, M. W. I.: Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions, Biogeosciences, 3, 397–420, doi:10.5194/bg-3-397-2006, 2006.

Riis, V., Lorbeer, H., and Babel, W.: Extraction of microorganisms from soil: Evaluation of the efficiency by counting methods and activity measurements, Soil Biol. Biochem., 30, 1573–1581, doi:10.1016/s0038-0717(97)00232-0, 1998.

Rumpel, C., Chaplot, V., Planchon, O., Bernadou, J., Valentin, C., and Mariotti, A.: Preferential erosion of black carbon on steep slopes with slash and burn agriculture, Catena, 65, 30–40, doi:10.1016/J.Catena.2005.09.005, 2006.

Schmid, E. M., Skjemstad, J. O., Glaser, B., Knicker, H., and Kogel-Knabner, I.: Detection of charred organic matter in soils from a Neolithic settlement in Southern Bavaria, Germany, Geoderma, 107, 71–91, Pii S0016-7061(01)00139-2, 2002.

Schmidt, M. W. I., Skjemstad, J. O., Gehrt, E., and Kogel-Knabner, I.: Charred organic carbon in German chernozemic soils, European Journal of Soil Science, 50, 351–365, 1999.

Schmidt, M. W. I. and Noack, A. G.: Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges, Global Biogeochem. Cycles, 14, 777–793, 2000.

Schmidt, M. W. I., Skjemstad, J. O., and Jager, C.: Carbon isotope geochemistry and nanomorphology of soil black carbon: Black chernozemic soils in central Europe originate from ancient biomass burning, Global Biogeochem. Cycles, 16, 1123, doi:10.1029/2002GB001939, 2002.

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., and Trumbore, S. E.: Persistence of soil organic matter as an ecosystem property, Nature, 478, 49–56, doi:10.1038/Nature10386, 2011.

Schneider, M. P. W., Hilf, M., Vogt, U. F., and Schmidt, M. W. I.: The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 degrees C and 1000 degrees C, Organic Geochemistry, 41, 1082–1088, doi:10.1016/J.Orggeochem.2010.07.001, 2010.

Schneider, M. P. W., Smittenberg, R. H., Dittmar, T., and Schmidt, M. W. I.: Comparison of gas with liquid chromatography for the determination of benzenepolycarboxylic acids as molecular tracers of black carbon, Organic Geochemistry, 42, 275–282, doi:10.1016/j.orggeochem.2011.01.003, 2011.

Seifritz, W.: Should We Store Carbon in Charcoal, International Journal of Hydrogen Energy, 18, 405–407, 1993.

Seiler, W. and Crutzen, P. J.: Estimates of Gross and Net Fluxes of Carbon between the Biosphere and the Atmosphere from Biomass Burning, Climatic Change, 2, 207–247, 1980.

Shindo, H.: Elementary composition, humus composition, and decomposition in soil of charred grassland plants Soil Science and Plant Nutrition, 37, 651–657, 1991.

Shneour, E. A.: Oxidation of graphitic carbon in certain soils, Science, 151, 991–992, doi:10.1126/science.151.3713.991, 1966.

Skjemstad, J. O., Clarke, P., Taylor, J. A., Oades, J. M., and McClure, S. G.: The chemistry and nature of protected carbon in soil, Australian Journal of Soil Research, 34, 251–271, 1996.

Skjemstad, J. O., Reicosky, D. C., Wilts, A. R., and McGowan, J. A.: Charcoal carbon in US agricultural soils, Soil Science Society of America Journal, 66, 1249–1255, 2002.

Smernik, R. J., Kookana, R. S., and Skjemstad, J. O.: NMR characterization of C-13-benzene sorbed to natural and prepared charcoals, Environ. Sci. Technol., 40, 1764–1769, doi:10.1021/Es051895o, 2006.

Smith, J. L., Collins, H. P., and Bailey, V. L.: The effect of young biochar on soil respiration, Soil Biol. Biochem., 42, 2345–2347, doi:10.1016/J.Soilbio.2010.09.013, 2010.

Spokas, K. A.: Review of the stability of biochar in soils: predictability of O:C molar ratios, Carbon Management, 1, 289–303, 2010.

Torn, M. S., Lapenis, A. G., Timofeev, A., Fischer, M. L., Babikov, B. V., and Harden, J. W.: Organic carbon and carbon isotopes in modern and 100-year-old-soil archives of the Russian steppe, Glob. Change Biol., 8, 941–953, 2002.

Trumbore, S.: Age of soil organic matter and soil respiration: Radiocarbon constraints on belowground C dynamics, Ecol. Appl., 10, 399–411, 2000.

Updegraff, K., Pastor, J., Bridgham, S. D., and Johnston, C. A.: Environmental and Substrate Controls over Carbon and Nitrogen Mineralization in Northern Wetlands, Ecol. Appl., 5, 151–163, 1995.

Vasilyeva, N. A., Abiven, S., Milanovskiy, E. Y., Hilf, M., Rizhkov, O. V., and Schmidt, M. W. I.: Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem, Soil Biol. Biochem., 43, 1985–1988, doi:10.1016/J.Soilbio.2011.05.015, 2011.

Westerling, A. L., Hidalgo, H. G., Cayan, D. R., and Swetnam, T. W.: Warming and earlier spring increase western US forest wildfire activity, Science, 313, 940–943, doi:10.1126/Science.1128834, 2006.

Zimmerman, A. R.: Abiotic and Microbial Oxidation of Laboratory-Produced Black Carbon (Biochar), Environ. Sci. Technol., 44, 1295–1301, doi:10.1021/es903140c, 2010.

Manuscript II

Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment

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Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment

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Abstract

Pyrogenic organic matter (PyOM) decomposes on centennial timescale in soils, but the processes regulating its decay are poorly understood. We conducted one of the first studies of PyOM and wood decomposition in a temperate forest using isotopically labeled organic substrate, and quantified microbial incorporation and physico-chemical transformations of PyOM *in situ*. Stable-isotope (^{13}C and ^{15}N) enriched PyOM and its precursor wood were added to the soil at 2 cm depth at ambient (N0) and increased (N+) levels of nitrogen fertilization. The carbon (C) and nitrogen (N) of added PyOM or wood were tracked through soil to 15 cm depth, in physically separated soil density fractions and in benzene polycarboxylic acids (BPCA) molecular markers. After 10 months *in situ*, more PyOM-derived C (>99% of initial ^{13}C-PyOM) and N (90% of initial ^{15}N-PyOM) was recovered than wood derived C (48% of ^{13}C-wood) and N (89% under N0 and 48% under N+). PyOM-C and wood-C migrated at the rate of 126 mm yr^{-1} with 3–4% of PyOM-C and 4–8% of wood-C recovered below the application depth. Most PyOM C was recovered in the free light fraction (fLF) (74%), with 20% in aggregate-occluded and 6% in mineral associated fractions – fractions that typically have much slower turnover times. In contrast, wood C was recovered mainly in occluded (33%) or dense fraction (27%). PyOM addition induced loss of native C from soil (priming effect), particularly in fLF (13%). The total BPCA-C content did not change but after 10 months the degree of aromatic condensation of PyOM decreased, as determined by relative contribution of benzene hexa-carboxylic acid (B6CA) to the total BPCA C. Soil microbial biomass assimilated 6–10% of C from the wood, while PyOM contributions was negligible (0.14–0.18%). The addition of N had no effect on the dynamics of PyOM while limited effect on wood.

Abbreviations

PyOM =	pyrogenic organic matter
BPCA =	benzene polycarboxylic acids
fLF =	free light fraction
oLF =	occluded light fraction
DF =	dense fraction
SOM =	soil organic matter
PE =	priming effect
SMB =	soil microbial biomass

Keywords: ^{13}C, ^{15}N, benzene polycarboxylic acids, density fractionation, microbial biomass, nitrogen deposition, priming effect, pyrogenic organic matter, soil organic matter

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Introduction

Pyrogenic organic matter (PyOM), a product of incomplete combustion of biomass (Goldberg, 1985), is ubiquitous in soils (Schmidt & Noack, 2000) and can account for up to 45% of total soil organic carbon (SOC) (Bird *et al.*, 1999; Schmidt *et al.*, 1999b; Skjemstad *et al.*, 2002; Lehmann *et al.*, 2008; Rovira *et al.*, 2009). In recent years, PyOM has received considerable interest by researchers, in part, because of its potential relevance to the carbon (C) cycle of terrestrial ecosystem (Schmidt *et al.*, 2011). Climate change projections predict an increase in the wildfire frequency and intensity in temperate and boreal regions (Westerling *et al.*, 2006; Moritz *et al.*, 2012), which would increase the inputs of PyOM to soils. The addition of PyOM to soils could also constitute a method for sequestering C (Lehmann *et al.*, 2006; Deluca & Aplet, 2008), if its residence time

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in soil were sufficiently longer than its precursor biomass. However, many uncertainties remain about the dynamics of PyOM C and nitrogen (N) in soils including *in situ* PyOM turnover rates, degradation pathways and stabilization mechanisms (Knicker, 2011; Singh *et al*., 2012).

Most wood decomposition studies estimate decay rates as either mass loss or density change per unit time (Chambers *et al*., 2001). These studies have estimated yearly to decadal turnover time for fine woody debris and litter (Abbott & Crossley, 1982; Busse, 1994; Clark *et al*., 2002; Guo *et al*., 2006; Zell *et al*., 2009; Fasth *et al*., 2011). Degradation rates of pyrolyzed wood are slower than its initial precursor biomass and becomes even slower with increasing pyrolysis temperatures (Baldock & Smernik, 2002). The slow decomposition of pyrolyzed wood, and pyrogenic matter in general, has been attributed to changes in physical and chemical structure, including an increase in the degree of aromatic condensation as the pyrolysis temperature increase from 200° to 600 °C (Baldock & Smernik, 2002; Keiluweit *et al*., 2010; Schneider *et al*., 2010; Zimmerman, 2010; Chatterjee *et al*., 2012).

In the last decade, studies on PyOM degradation in soils based on field (Bird *et al*., 1999; Hammes *et al*., 2008; Nguyen *et al*., 2008) and incubation studies (Baldock & Smernik, 2002; Hamer *et al*., 2004; Kolb *et al*., 2009; Kuzyakov *et al*., 2009; Hilscher & Knicker, 2011; Santos *et al*., 2012) challenges the view that PyOM persists in soils for millennia (Schmidt & Noack, 2000). A recent synthesis of current knowledge of PyOM degradation in soil estimated turnover times on centennial scales (Singh *et al*., 2012). In addition to losses via mineralization, PyOM is also vertically mobile in mineral soil (Skjemstad *et al*., 1999; Dai *et al*., 2005; Rumpel *et al*., 2006; Brodowski *et al*., 2007) and can eventually be lost from soil by leaching (Shinogi *et al*., 2003; Hockaday *et al*., 2006; Cheng & Lehmann, 2009; Major *et al*., 2009b; Abiven *et al*., 2011). PyOM dissolution from soils could therefore be an important translocation mechanism in the terrestrial system.

Recent research on soil organic matter (SOM) concludes that long-term persistence of SOM, that is, on centennial scales, depends on its inaccessibility to microorganisms, such as through organo-mineral interactions (Von Lützow *et al*., 2006; Schmidt *et al*., 2011). PyOM interacts with the soil mineral phase (Glaser *et al*., 2000; Brodowski *et al*., 2006; Cheng *et al*., 2006; Cheng & Lehmann, 2009) and has been posited to act as a binding agent for soil aggregates (Brodowski *et al*., 2005a). Vasilyeva *et al.* (2011) observed that 70% of PyOM was associated with the dense fraction (>2 g cm^{-3}) under 55 years of fallow management (fire suppression) in the Streletzkaya steppe (Russia), and suggested its stabilization by clay micro-aggregation. These studies indicate that aggregation and mineral interactions may be important mechanisms for stabilizing PyOM in soil. However, we do not know when and to what extent such stabilizing mechanisms occur during the PyOM decomposition pathway.

Atmospheric N deposition has increased in recent decades and is predicted to further rise (Galloway *et al*., 2008). This is important because C and nitrogen (N) cycles are closely linked at all scales and interact in many ways and therefore cannot be considered separately (Norby, 1998). N addition has decreased (Fog, 1988; Turunen *et al*., 2004; Waldrop *et al*., 2004; Janssens *et al*., 2010), increased (Mack *et al*., 2004; Waldrop *et al*., 2004; Bragazza *et al*., 2006), or had no effect on (Knorr *et al*., 2005) SOM decomposition rates. There are fewer studies on the effects of increased N on wood and PyOM decomposition. These studies observed increased (Micks *et al*., 2004; Wal *et al*., 2007; Allison *et al*., 2009; Bebber *et al*., 2011) or level (Mccoll & Powers, 1998) decomposition rates for wood, with no effect on PyOM (Santos *et al*., 2012).

Recent observations showed that C mineralization rates for native SOM could be influenced from PyOM addition to mineral soils (i.e. priming effect). However, the direction of this priming effect is under debate. PyOM has been shown to inhibit (negative priming) (Jones *et al*., 2011), have no effect (Hilscher *et al*., 2009; Kolb *et al*., 2009; Kuzyakov *et al*., 2009; Abiven & Andreoli, 2010; Santos *et al*., 2012), or increase native SOM mineralization rates (positive priming) (Steinbeiss *et al*., 2009; Zimmerman *et al*., 2011). Moreover, in an incubation study using different types of PyOM, Zimmerman *et al*. (2011) observed a change in priming effects from positive to negative with time. Several mechanisms have been proposed to explain these contradictory priming effects associated with added PyOM, including (i) differences in the physicochemical state of PyOM in different soils (Santos *et al*., 2012), (ii) encapsulation and/or sorptive protection of SOM by PyOM (Zimmerman *et al*., 2011), (iii) nutritional competition and balance between r and K strategist microbial population (Fontaine *et al*., 2003), or (iv) changes in microbial community structure (Blagodatskaya & Kuzyakov, 2008). For wood-amended soil, only a few studies reported positive priming effects on SOM mineralization (Sulzman *et al*., 2005; Crow *et al*., 2009) or no priming effect (Santos *et al*., 2012).

No reported field studies have compared the C and N dynamics of PyOM and its precursor biomass during its decomposition in soil. To evaluate the potential of PyOM as a long-term C sink in soil as compared to its precursor biomass, we conducted a field study to quantify the fate of added $^{13}C/^{15}N$ labeled PyOM and its

precursor biomass (*Pinus ponderosa* wood) to a forest soil during 10 months, *in situ*. To determine the effect of added inorganic N on PyOM and wood dynamics in soil, we experimentally manipulated N deposition to half of the plots by adding ammonium nitrate (NH_4NO_3) at (+60 kg N ha^{-1} yr^{-1}). The objectives of our study were to (i) determine recovery of C and N from PyOM and its precursor wood in soil (0–15 cm) after 10 months; (ii) quantify the vertical movement of PyOM and wood C and N in the soil profile; (iii) investigate the main stabilization mechanism for PyOM and/or wood by determining the partitioning of PyOM and wood C and N within operational-defined SOM fractions; (iv) determine chemical changes in PyOM, both in quality and quantity in comparison to initial input, and (v) assess the effect of N treatment on the decomposition dynamics of PyOM and wood in soil.

Materials and methods

Field site

The experimental site is a mixed, beech-dominated temperate forest, located 20 km northwest of Zurich, CH (47°28′40.8′N, 8°21′55.2′E) on the south-facing slope of Lägeren Mountain (eastern-most part of the Jura Mountain range) at 680 m above sea level. The mean annual temperature is 8.4 °C, and mean annual precipitation is 930 mm (Ruehr & Buchmann, 2010). The soil at the site is classified as a Cambisol (F.A.O.-U.N.E.S.C.O., 1998). Chemical and physical properties of the soil (0–10 cm) are presented in Table 1. Soil volumetric moisture content and soil temperature were monitored every 30 min at two depths (5 cm and 10 cm below the surface) within each field replicates, using soil moisture temperature sensors (ECH2O-TE/EC-TM, Decagon Devices, Pullman, WA, USA) connected to a data-logger. Soil moisture in the field site ranged from 20 to 50%, while temperatures ranged from 0 to 25 °C (at 5 cm depth) during the first year of the study.

Experimental design

The experiment is located within a forest gap created by a natural windthrow (in 1999), which has been subsequently mowed to maintain open conditions. The site was chosen to provide similar micro-climatic conditions to a post-fire gap. The experimental setup was a randomized block design, having the factorial combination of three types of organic inputs (i.e., pinewood, PyOM, and no input as control) and two treatment levels of N (ambient = N0 and added N = N+) with three field replicates (n = 3) per treatment combination. The ambient, natural N deposition at the field site was estimated to be 20 kg N yr^{-1} ha^{-1} (Kloeti *et al.*, 1989). The N+ treatment corresponds to a level of ca. 80 kg N yr^{-1} ha^{-1} (60 kg N yr^{-1} ha^{-1} added to the ambient N deposition). The wood used for the study was primary stem biomass from two-year-old *Pinus ponderosa* saplings grown under controlled

Table 1 Physical and chemical characteristics of the soil in the 0–15 cm depth. Values correspond to the mean (n = 3) and values between brackets correspond to SE

Texture %			Bulk density g cm^{-3}				CEC	Elemental analysis g kg^{-1} soil										
Sand	Silt	Clay	0–5 cm	5–10 cm	10–15 cm	pH	mmol kg^{-1}	C	H	N	Na	Mg	Al	Si	P	K	Ca	Mg	Fe
45.5	24.2	31.5	1.20	1.21	1.60	5.9	74.3	33.7	8.9	2.4	7.8	11.2	71.6	317	0.3	19.5	4.6	1.4	32.8
(2.0)	(2.5)	(1.4)	(0.1)	(0.2)	(0.2)	(0.3)	(8.6)	(2.8)	(0.4)	(0.1)									

greenhouse conditions and labeled with $^{13}CO_2$ and $^{15}NO_3^-$ (Bird & Torn, 2006). PyOM was obtained by charring the labeled wood at 450 °C for 5 h under N_2 flux according to Hammes *et al.* (2006). The chemical characteristics of both labeled wood (^{13}C = 2.05 atom% and ^{15}N = 4.3 atom%) and PyOM (^{13}C = 2.03 atom% and ^{15}N = 4.2 atom%) are described in Santos *et al.* (2012). The C and N elemental composition of wood was 499 g kg^{-1} and 4.3 g kg^{-1}, respectively. For PyOM, C concentration was 799 g kg^{-1} and N was 7.1 g kg^{-1}. The structure of the PyOM and wood is detailed by magnetic resonance, mid-infrared spectroscopy and mass spectrometry in Chatterjee *et al.* (2012). The wood and PyOM were uniformly labeled (Yarnes *et al.*, 2011; Santos *et al.*, 2012). Both wood and PyOM were ground (<2 mm) prior to soil addition.

In each plot, we inserted 20 cm long and 10 cm diameter mesocosms (polyethylene tubes, smoothed at the top and sharpened at an angle at the bottom) into the soil up to a depth of 15 cm from the surface. Each mesocosm had two 4 cm diameter windows (at 7.5 cm and 12 cm distance from the bottom and aligned at 120º to one another), fit with 0.7 mm stainless steel mesh to allow fungal hyphae and some fine roots to penetrate the core and limit lateral movement of the added substrate (Bird & Torn, 2006). Mesocosms were placed >1 m from large trees and >0.5 m from the adjacent mesocosms. In April 2009, the mesocosms were installed at the field site and allowed to equilibrate for 180 days before the addition of the organic inputs.

In October 2009, ^{13}C and ^{15}N enriched-wood and PyOM were applied to the mesocosms at a rate of 189 g C m^{-2} for wood or 397 g C m^{-2} for PyOM, at 2 cm soil depth and mixed gently with 1–2 mm of mineral soil. The PyOM application rate was based on a previous estimate of PyOM inputs to soil after a fire in a similar forest type (Eckmeier *et al.*, 2007b). The amount of wood was based on estimation of twig and other wood contribution to the litter (Kammer & Hagedorn, 2011; Jones *et al.*, 2011). Unamended-control mesocosms were similarly disturbed to those that received wood or PyOM. Beginning in March 2010, 11.4 mg of $NH_4^+NO_3^-$ dissolved in 10 ml of water was added monthly for 10 months (equivalent to 60 kg N ha^{-1} yr^{-1}) to increased N (N+) treatment mesocosms, while an equivalent amount of distilled water was added to ambient N (N0) treatment mesocosms.

Soil sampling and analysis

We sampled the intact mesocosms (n = 18) 10 months after the PyOM or wood additions to the soil mesocosms. The soil within the mesocosms was separated immediately into 0–5 cm, 5–10 cm, and 10–15 cm depth. Soil fauna, stones (>2 mm), and roots (>2 mm) were manually removed and stored separately. Soil subsamples were air-dried and ball-milled for physico-chemical analysis. Soil water content was determined by drying 1 g of soil (n = 3) at 105 °C for 24 h. Microbial biomass analysis (chloroform fumigation extraction method) was performed immediately after sampling on fresh soil (Vance *et al.*, 1987). Total C and N contents in soil samples were determined with a CHN elemental analyzer (EA 1108; Carlo Erba, Cornaredo, Italy). The soil pH values were measured on air-dried soil at mass-to-volume ratio of 1 : 2.5 (soil : water ratio) (Jackson, 1958).

Soil organic matter fractionation

We used a density fractionation approach to partition SOM into three main pools that differ in their main stabilization mechanisms and turnover times. For each fraction, we quantified the ^{13}C and ^{15}N excess from added PyOM or wood to assess the distribution of PyOM and wood into discrete physical fractions as a means for identifying mechanisms such as organo-mineral interactions. In our study, the free light fraction (fLF) was separated using a density of 1.6 g cm^{-3} (Glaser *et al.*, 2000; Cerli *et al.*, 2012) and the occluded light fraction (oLF) was separated after gentle ultrasonic dispersion using a sonifier (Bandelin Sonoplus HD 3400; Berlin, Germany; calibrated according to Schmidt *et al.* (1999a). We applied 250 J ml^{-1} of disruptive energy per sample. This rate was based on analysis of oLF yield and C content across a dispersive energy range (0–300 J ml^{-1}, data not shown). For the density fractionation, a subsample (10 g) of air-dried sample (0–5 cm) was suspended in 50 ml of 1.6 g cm^{-3} sodium polytungstate (SPT) solution (TC-Tungsten compounds), the suspension was allowed to settle for 1 h and centrifuged (3237 *g*, 30 min; Heraeus Megafuge 1.0, Newport Pagnell, UK). The floating material (≤1.6 g cm^{-3}) was collected as fLF on a glass microfiber filters with 1.5 μm particle retention (934-AH, Whatman, Maidstone, UK), washed thoroughly with deionized water to remove any SPT (conductivity of supernatant water <50 μS cm^{-1}) and freeze-dried. The remaining pellet for each sample was resuspended in SPT and treated with ultra sonification (250 J ml^{-1}) to destroy aggregates. The suspension was allowed to settle for 4 h, followed by centrifugation (3237 *g*, 30 min). The oLF (<1.6 g cm^{-3}) was collected similarly as above on glass microfiber filters and washed thoroughly with deionized water (conductivity of supernatant water <50 μS cm^{-1}). The remaining dense fraction (DF) was washed until the SPT was removed completely (conductivity of supernatant water <50 μS cm^{-1}). The DF was not further separated via physical fractionation into sand or clay/silt fractions. It is considered a heterogeneous mixture of organic matter in different types of association with minerals (i.e., denser than 1.6 g cm^{-3}). Some part of organic matter present in DF could be uncomplexed organic matter in sand sized separates. The density fractions (fLF, oLF, DF) were ball-milled to homogenize the samples. C and N concentration was measured with an elemental analyzer (EA 1108; Carlo Erba) and ^{13}C and ^{15}N was measured using an isotope ratio mass spectrometer (IRMS) (Delta S, Thermo Finnigan, MAT, Bremen, Germany). The recovery of C and N was calculated based on the amount of C and N present in 0–5 cm depth soil after 10 months.

Benzenepolycarboxylic acid (BPCA) analysis

The BPCA molecular marker method was employed to quantify and characterize the PyOM before and after its addition to the soil (Glaser *et al.*, 1998; Brodowski *et al.*, 2005b; Schneider *et al.*, 2010). Subsamples (400–500 mg) of PyOM-amended

air-dried soil (0–5 cm, $n = 3$) and PyOM (30–40 mg) were pre-treated with 4 M trifluroacetic acid (4 h, 105 °C) to remove Fe and Al, followed by conversion of PyOM into BPCAs by nitric acid oxidation (8 h, 170 °C). The digested extract was further purified using cation-exchange resin to remove any polyvalent cations. The extracts were freeze-dried and subsequently derivatized into trimethylsilyl derivatives to be analyzed on a gas chromatograph (Agilent 6890, Palo Alto, CA, USA) equipped with a flame ionization detector and a DB-5MS capillary column (50 m × 0.2 mm i.d., 0.33 μm film thickness). Each analysis was performed in triplicate. The acids with 3, 4, 5, and 6 carboxyl functions (B3CA, B4CA, B5CA, and B6CA, respectively) were identified and summed up to represent the total amount of pyrogenic molecular markers in the PyOM.

Microbial biomass by chloroform fumigation direct extraction

Moist soil, equivalent of 20 g of oven-dried soil (105 °C, 24 h), was fumigated with alcohol free chloroform in desiccators for 24 h in the dark (Vance *et al*., 1987). The fumigated soil and an equivalent amount of non-fumigated soil for each sample was then extracted using 1 M KCl (1 : 5 soil solution ratio) for 1 h, filtered (Whatman GF 934-AH; Whatman), and extracts stored at –20 °C until analysis. The total organic C (TOC) in fumigated and non-fumigated extracts were analyzed using a TOC analyzer (TOC-V; Shimadzu Corporation, Kyoto, Japan). A conversion factor of 0.45 (K_c) (Wu *et al*., 1990) and 0.68 (K_n) (Shen *et al*., 1984) was applied for incomplete extraction for microbial C and N, respectively. PyOM could adsorb lysed cells and may influence microbial biomass recovery (Durenkamp *et al*., 2010; Liang *et al*., 2010). Nevertheless, the amount of PyOM C contributing to total SOC is inversely correlated to the extraction efficiency (Liang *et al*., 2010). In this study, the applied PyOM C contributed to 11% of total SOC and therefore, adsorption of lysed microbial biomass on PyOM is assumed to be negligible. The $\delta^{13}C$ and $\delta^{15}N$ of extracts were analyzed on freeze-dried extracts using IRMS (Delta S, Thermo Finnigan) (Murage & Voroney, 2007).

The $\delta^{13}C$ (‰) of soil microbial biomass C (SMB-C) was estimated as the $\delta^{13}C$ of the C extracted from the fumigated sample in excess of that extracted from the non-fumigated sample, using Eqn (1) (Murage & Voroney, 2007),

$$\delta^{13}C = \frac{(\delta^{13}C_f \times C_f) - (\delta^{13}C_{nf} \times C_{nf})}{(C_f - C_{nf})} \quad (1)$$

where C_f and C_{nf} were the amounts of C extracted from the fumigated and non-fumigated samples (μg C g^{-1} dry soil) and $\delta^{13}C_f$ and $\delta^{13}C_{nf}$ were the ^{13}C natural abundance of the fumigated and non-fumigated extracts (%), respectively.

The soil under no treatment (control plots) was taken as the reference, and the proportion of labeled substrate-derived-C in SMB was calculated using Eqn (2),

$$f_{C-\text{substrate}}, \% = \left(\frac{\delta_{\text{treated}-\text{microbes}} - \delta_{\text{control microbes}}}{\delta_{\text{substrate}} - \delta_{\text{control microbes}}}\right) \quad (2)$$

where $\delta_{\text{treated-microbes}} = \delta^{13}C$ value of SMB extracted from the substrate-treated mesocosms, $\delta_{\text{substrate}} = 842‰$ for wood and 800‰ for PyOM, and $\delta_{\text{control microbes}} = \delta^{13}C$ the value of SMB extracted from the control mesocosms.

Calculations

The amount of labeled substrate-C (or N) (PyOM and wood) recovered in the bulk soil and density fractions was calculated for each mesocosms using a two end-member linear mixing model Eqns (3) and (4) (Bernoux *et al*., 1998),

$$f_{\text{substrate}} = \frac{\delta_{\text{soil sample}} - \delta_{\text{control soil}}}{\delta_{\text{substrate}} - \delta_{\text{control soil}}} \quad (3)$$

$$\text{Amount of substrate}_{C\,or\,N}, \% = C \text{ or } N_{\text{sample}}(\%) \times f_{\text{substrate}}, \quad (4)$$

where $f_{\text{substrate}}$ is the fraction of substrate in the soil, $\delta_{\text{soil sample}}$, $\delta_{\text{control soil}}$, $\delta_{\text{substrate}}$ is isotopic value (either $\delta^{13}C$ or $\delta^{14}N$, ‰) of soil sample, corresponding control soil within field replicates, and substrate (PyOM or wood), respectively. *C (or N)* is the amount of C (or N) in% of the soil sample.

To calculate excess ^{13}C or ^{15}N values in soil and microbial biomass, we used Eqn (5) (Dawson *et al*., 2002),

$$\text{Excess } ^{13}C \text{ or } ^{15}N = \frac{\text{atom}\%_{\text{sample}} - \text{atom}\%_{\text{background}}}{100} \times \text{C or N} \quad (5)$$

where Excess ^{13}C or ^{15}N (μg g^{-1} dry soil for microbes) is the total amount of ^{13}C or ^{15}N added by labeled PyOM or wood to soil or microbial biomass, $\text{atom}\%_{\text{sample}}$ is the atom % of soil or microbial biomass in substrate-treated sample, and $\text{atom}\%_{\text{background}}$ is the atom% of soil or microbial biomass in the control treatments averaged over three plots. C or N is the total organic C or N content of soil (g kg^{-1} soil) or microbial biomass (μg g^{-1} dry soil).

To estimate the potential migration rate of substrate-C in the soil profile, we use Eqn (6)

$$\text{Migration rate}_{\text{input}-C} = \frac{d_{max}}{t} \quad (6)$$

where d_{max} (mm) is the maximum depth where PyOM was recovered below its application depth after time t (in years). We estimated d_{max} as 10.5 cm based on the recoveries of PyOM and wood at depth 10–15 cm (average of 12.5 cm as soil was homogenized) and application depth at 2 cm below the surface.

Statistical analysis

We performed an analysis of variance (ANOVA) using SPSS (IBM-SPSS statistics 20.0 package for Mac, Armonk, NY, USA) to determine the effect of the different input treatments and the two levels of N application, and the interactions between the two factors at different depths (0–5 cm, 5–10 cm, and 10–15 cm). Differences in the relative contribution of individual molecular markers and BPCA-C (g kg^{-1} OC) at $t = 0$ and

t = 10 months were tested with independent t-test, when the data were normally distributed (Shapiro–Wilk test), and non-parametric Mann–Whitney U-test when the normality test failed. Levene's test for equality of variance was used to determine homogeneity in the data, and significance test was used accordingly. We considered differences between treatments with $P \leq 0.05$ as significant.

Results

Total C and N and recovery of substrate-C or N in the topsoil

We observed no change in soil total C in mesocosms (0–15 cm) due to different organic inputs or N ($P > 0.05$, $n = 3$), except in wood-amended soil under the N+ treatment which had a significantly higher total C than N0 at 0–5 cm depth ($P = 0.007$). Including all depth and across all N treatments, we recovered 99.6 ± 0.2% of the initial ^{13}C-PyOM and 48.3 ± 4.4% of the initial ^{13}C-wood after 10 months (Table 2). Most of the ^{13}C-PyOM (95 ± 1.7% for N0 and 96.5% ±1.4% for N+) and ^{13}C-wood (40.0 ± 13.2% under N0 and 44.5 ± 1.4% under N+) were recovered between 0 and 5 cm. Both PyOM-C and wood-C were recovered at the depth 10–15 cm, indicating vertical movement with a migration rate of 126 mm yr^{-1} and 3–4% of PyOM-C and 4-8% of wood-C migrated below the incorporation depth of 2 cm (Table 2). Given the three depth intervals of sampling, the migration rate of PyOM and wood C could range between 100 mm yr^{-1} to 150 mm yr^{-1}. We observed no effect of the N treatment on either on the vertical movement (amount or rates) or C recovery of wood or PyOM.

Similarly, total soil N in mesocosms was not affected by added PyOM, wood or from added N. We recovered >90% of applied ^{15}N-PyOM, mainly at the application depth (0–5 cm; Table 2). The recovery of ^{15}N-PyOM across all N treatment (93.6 ± 2.2%, $n = 6$) in mesocosms was significantly lower than ^{13}C-PyOM ($P \leq 0.05$). More wood-^{15}N was recovered in mesocosms under ambient N levels (88.5 ± 6.4%) than with elevated (N+) levels (48.0 ± 6.0%, Table 2).

Soil organic matter fractions

The average recovery of C and N of bulk soil across all treatments ($n = 18$) after density fractionation was 88.8 ± 2.9% of total C and 84.8 ± 2.6% of the total N, respectively (Table 3). The loss of C and N is due to mobilization into SPT solution or as dissolved organic C and N during washing of density fractions with water to remove SPT, which were discarded during the process. After density fractionation, we recovered on average all of ^{13}C-PyOM (100 ± 3.7%, $n = 6$) and ^{15}N-PyOM (100 ± 6%, $n = 6$), but recovery of ^{13}C-wood (72.0 ± 9.7%; $n = 6$) and ^{15}N-wood (70 ± 14%; $n = 6$) was highly variable. The recovery of ^{13}C-wood was significantly lower than ^{13}C-PyOM ($P \leq 0.05$), while ^{15}N-wood recovery was highly variable among replicates, leading to no significant difference in ^{15}N-PyOM recoveries. The recoveries of PyOM and wood C and N are expressed as% of the amount recovered in the bulk soil between 0 and 5 cm depth after 10 months.

For all treatments, most of the SOC was in the DF. The C concentration and C : N ratios of density fractions increased in the order DF < fLF < oLF (Table 4). Relative to the unamended control, the C : N ratio of both PyOM and wood-amended soil was significantly higher ($P \leq 0.05$) in fLF and oLF density fractions, while DF had similar values to the control soils across

Table 2 Recovery of applied ^{13}C/^{15}N-labeled wood or PyOM and excess ^{13}C and ^{15}N in the soil at depths of 0–5 cm, 5–10 cm, and 10–15 cm and bulk soil, 10 months after application. The initial C : N ratio of added PyOM was 110 and wood was 115. Different letters in the same column (within the same depth) are significantly different ($P \leq 0.05$)

	Soil depth				Excess ^{13}C, mg kg^{-1} soil		
Substrate	0–5 cm	5–10 cm	10–15 cm	Bulk soil	0–5 cm	5–10 cm	10–15 cm
^{13}C-substrate recovered (% of the applied after 10 months)							
Wood, N0	40.0 (13.2)^{a}	6.5 (3.9)^{a}	1.6 (0.9)^{a}	48.2 (4.6)^{a}	14.7 (5.3)	2.0 (1.1)	0.5 (0.3)
Wood, N+	44.5 (5.1)^{a}	2.1 (0.3)^{a}	1.8 (0.9)^{a}	48.4 (8.8)^{a}	14.5 (3.0)	0.7 (0.1)	0.5 (0.2)
PyOM, N0	95.1 (1.7)^{b}	3.1 (0.9)^{a}	1.1 (0.7)^{a}	99.4 (0.3)^{b}	64.0 (8.5)	1.5 (0.3)	0.5 (0.2)
PyOM, N+	96.5 (1.4)^{b}	2.3 (0.7)^{a}	1.1 (0.9)^{a}	99.9 (0.4)^{b}	71.2 (5.8)	1.5 (0.5)	0.5 (0.4)
^{15}N-substrate recovered (% of the applied after 10 months)					Excess ^{15}N mg kg^{-1} soil		
Wood, N0	78.2 (3.8)^{a}	7.1 (2.8)^{a}	3.1 (0.8)^{a}	88.5 (6.4)^{a}	0.9 (0.05)	0.1 (0.02)	0.03 (0.01)
Wood, N+	35.1 (7.5)^{b}	7.8 (0.8)^{a}	5.1 (2.0)^{a}	48.0 (6.0)^{b}	0.4 (0.12)	0.1 (0.00)	0.05 (0.03)
PyOM, N0	81.5 (2.6)^{a}	6.1 (2.0)^{a}	2.0 (1.0)^{a}	89.2 (0.4)^{a}	2.0 (0.32)	0.1 (0.04)	0.03 (0.02)
PyOM, N+	89.2 (2.3)^{a}	5.8 (0.9)^{a}	2.7 (0.2)^{a}	97.7 (3.0)^{a}	2.4 (0.21)	0.6 (0.46)	0.05 (0.01)

Table 3 Total C and N recovery of bulk soil and added substrate after density fractionation. Values correspond to the mean (n = 3) and values between brackets correspond to SE

	Total C recovery, %		Total N recovery, %	
Treatments	Bulk soil	Added substrate	Bulk soil	Added substrate
Wood, N0	88.6 (18)	78.8 (1)	93.0 (17)	45.2 (14)
Wood, N+	91.4 (4)	67.4 (17)	85.0 (5)	94.7 (14)
PyOM, N0	88.4 (1)	102.5 (10)	83.0 (2)	103.9 (10)
PyOM, N+	90.4 (3)	106.3 (2)	85.2 (2)	97.8 (9)
Control, N0	86.1 (7)		85.5 (2)	
Control, N+	88.2 (3)		77.6 (5)	

all treatments. The important contribution of PyOM (22% of total C in fLF and 7% of total C in oLF was PyOM C) and wood (5% of total C in fLF and 3% of total C in oLF-C was wood C) in these fractions explains their higher C : N ratio. We did not observe any significant effects of N treatment on the C : N ratios in density fractions across treatment.

The distribution of PyOM-C and wood-C among the density fractions after 10 months of their application was not similar (Fig. 1). Under ambient N, PyOM-C was recovered on average (n = 3) mostly in the fLF (70.2 ± 4.8%) followed by oLF (22.7 ± 3.9%), while wood C was recovered mostly in oLF (42.6 ± 21.5%) and fLF (39.4 ± 20.8%). The DF showed least recovery for both organic-input C under ambient N but wood C (14.6 ± 9.4%) recovery in DF was double as compared to PyOM C (7.1 ± 1.2%). A similar trend was observed in the increased N treatment for PyOM C with 77.4 ± 2.2% in fLF, 17.3 ± 1.9% in oLF and 5.4 ± 0.9%. Wood C under increased N treatment, however, showed maximum recovery in the DF (38.6 ± 5.4%) followed by fLF (38.0 ± 11.5%) and oLF (23.3 ± 9.3%). Added N resulted in significantly higher wood C recovery in DF as compared to wood C recovery under ambient N (F = 9.6, P = 0.02) and PyOM C recovery under increased N treatment (F = 18.3, P = 0.003).

In PyOM-amended soils, across all N treatments (n = 6) we observed a decrease of 13 ± 3% in fLF, 4 ± 3% in oLF and 0.2 ± 0.3% in DF in native soil C concentration (priming effect – Fig. 2). Native soil C concentration in wood-amended soils did not show a significant change in fLF (4.5 ± 4.6%, P = 0.061) or oLF (3.3 ± 3.5%, P = 0.068). Under the added N treatment, wood-amended soil showed a significant increase in the DF-C (0.7 ± 0.3%, P = 0.04) and bulk soil (1.3 ± 0.3%, P = 0.01) with respect to zero. Added N did not affect native SOC content or interact with PyOM effects on SOC content (Fig. 2). Native soil organic N showed little modification in either wood or PyOM-amended soil.

PyOM quality and quantity using BPCA marker molecules

The BPCA-C content of the labeled PyOM used in this study was 145.9 ± 6.8 g BPCA-C kg^{-1} OC (without using any conversion factor) and is similar to standard PyOM produced at 450 °C (Schneider *et al*., 2010). The BPCA-C content of the labeled PyOM mixed with soil (1:137 PyOM: dry soil mass ratio that corresponds to the application rate at 0–5 cm depth) at time t = 0 was 28.7 ± 2.6 g BPCA-C kg^{-1} OC. Ten months after PyOM addition to soils, total BPCA-C content in the 0–5 cm depth was not significantly different than at t = 0 (28.5 ± 2.6 g BPCA-C kg^{-1} OC for N0 and 23.7 ± 14 g BPCA-C kg^{-1} OC for N+, P > 0.05).

We observed a significant increase in benzene tetra-carboxylic acids (B4CA, P = 0.004) and a significant decrease in benzene hexa-carboxylic acids (B6CA, P = 0.023), and therefore a shift in the relative contribution of individual molecular markers after 10 months (Fig. 3). We did not observe any significant change in

Table 4 Total yield (% of soil), total C and N in SOM fractions (g kg^{-1} fraction) and bulk soil (g kg^{-1} soil) from 0 to 5 cm depth. Values correspond to the mean (n = 3) and values between brackets correspond to SE

	Density Fractions (g cm^{-3})										
	fLF			oLF			DF			Bulk soil	
Treatments	Yield	C	N	Yield	C	N	Yield	C	N	C	N
Wood, N0	1 (0.1)	299 (21)	9 (0.8)	1 (0.3)	393 (17)	12 (0.5)	98 (0.3)	20 (2)	2 (0.1)	30 (5)	2 (0.3)
Wood, N+	3 (0.2)	296 (14)	10 (0.5)	2 (0.3)	404 (8)	14 (0.5)	99 (0.5)	32 (2)	2 (0.2)	48 (2)	3 (0.2)
PyOM, N0	2 (0.5)	394 (19)	8 (1.6)	2 (0.3)	421 (27)	13 (2.2)	96 (0.9)	21 (2)	2 (0.2)	41 (6)	2 (0.3)
PyOM, N+	3 (0.2)	387 (32)	8 (0.1)	3 (0.3)	428 (5)	14 (0.5)	95 (0.5)	23 (3)	2 (0.2)	45 (3)	3 (0.2)
Control, N0	1 (0.2)	288 (28)	11 (0.3)	2 (0.1)	393 (9)	15 (0.3)	97 (0.2)	24 (2)	2 (0.2)	36 (4)	2 (0.2)
Control, N+	1 (0.2)	297 (13)	11 (0.3)	2 (0.3)	405 (1)	16 (0.5)	97 (0.5)	24 (3)	2 (0.1)	36 (2)	3 (0.1)

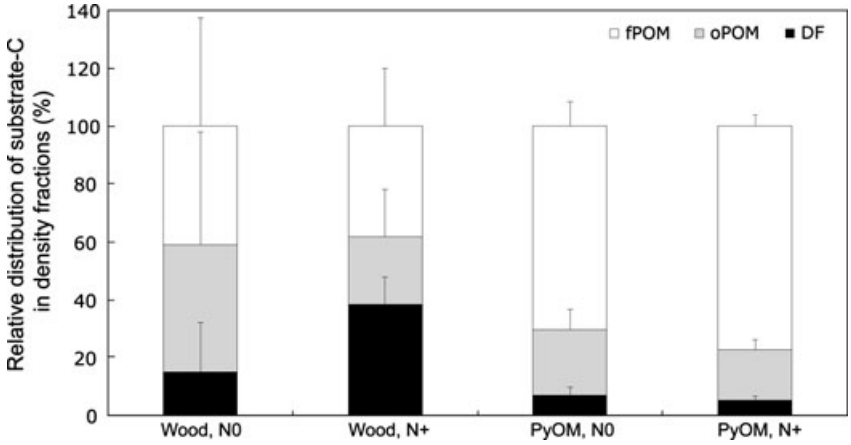

Fig. 1 Distribution of ^{13}C excess derived from wood or PyOM among soil organic matter (SOM) fractions (fLF: free light fraction; oLF: occluded light fraction and DF: dense fraction) 10 months after organic substrates application to the soil. SOM fractions shown are from 0 to 5 cm soil depth. The values correspond to the mean (n = 3) and the bars to the SE.

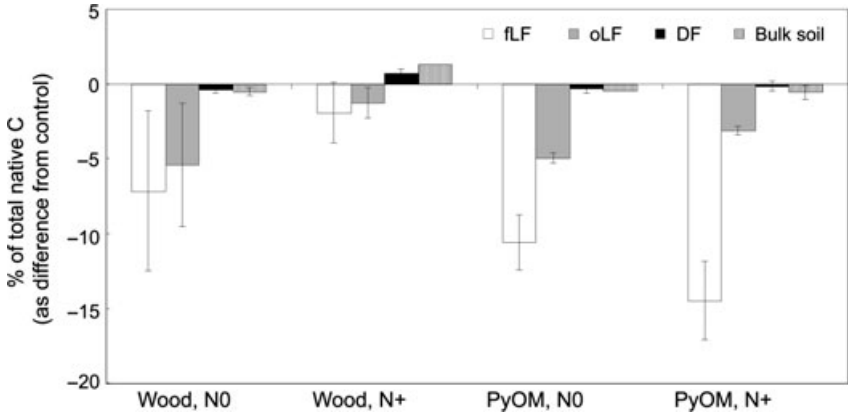

Fig. 2 Relative changes of native SOC among SOM fractions (fLF, oLF and DF) and bulk soils 10 months after addition of $^{13}C/^{15}N$-labeled wood or PyOM, expressed as differences from control plots. The error bars represent SE, n = 3.

the relative proportion of either benzene tricarboxylic acids (B3CA) or benzene penta-carboxylic acid (B5CA) and no significant effect of added N on BPCA distribution patterns.

Microbial C and N

Microbial biomass C (μg per g dry soil) was unaffected by addition of wood or PyOM after 10 months in the mesocosms (Table 4). Microbial biomass C declined significantly with increasing depths (0–5, 5–10 and 10–15 cm) averaged across all treatments ($P < 0.05$). ^{13}C-wood contributed 6–10% of microbial biomass, two levels of magnitude higher than from ^{13}C-PyOM (between 0.14 and 0.18% PyOM-^{13}C).

Microbial biomass N (μg per g dry soil) decreased with increasing depth from 0 to 5 cm to 10 cm to 15 cm (Table 4), except for wood-amended soil under N0 treatment. In the 0–5 cm depth, microbial biomass N increased ($P = 0.09$) with added N in wood-amended soil (51 ± 17 μg N g^{-1} dry soil under N0 and 89 ± 5 μg N g^{-1} dry soil under N+), while PyOM showed an opposite trend (94 ± 51 μg N g^{-1} dry soil under N0 and 64 ± 30 μg N g^{-1} dry soil under N+) but it was not significant (Table 5). Despite the highly labeled material used in this study, the ^{15}N signal was not detectable from added PyOM.

Discussion

Loss of PyOM and wood by decomposition and downward migration in soil

We recovered >99% PyOM-C but only half of wood-C in the soil mesocosms after 10 months *in situ* averaged across all treatments, which is consistent with the

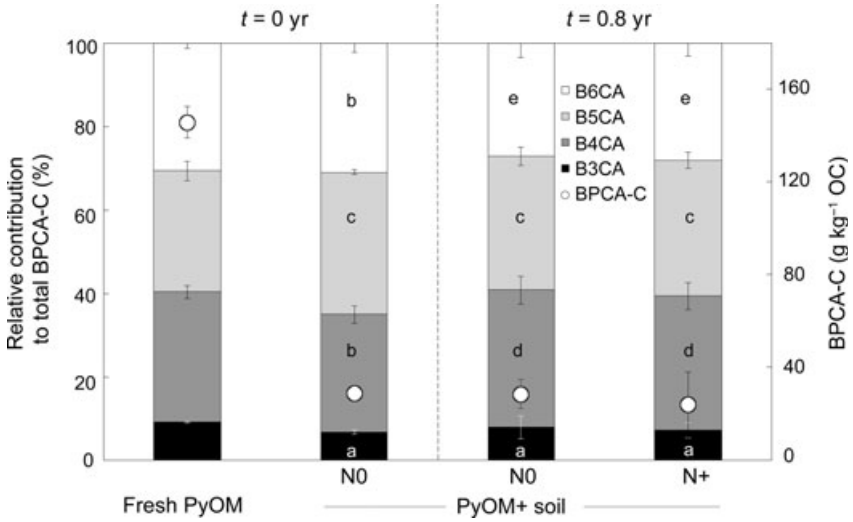

Fig. 3 Relative contributions of individual benzene polycarboxylic acids (BPCA) marker molecules (left, *y*-axis as bars) and total BPCA-C contents (right, *y*-axis as circles) in fresh PyOM, PyOM mixed with soil before (t = 0) and 10 months after the experiment started under ambient N (N0) and increased N (N+) treated soils. Different letters indicate significance difference ($P < 0.05$). The values correspond to the mean (n = 3) and the bars to the SE; B3CA, benzene tri-carboxylic acid; B4CA, benzene tetra-carboxylic acid; B5CA, benzene penta-carboxylic acid; B6CA, benzene hexa-carboxylic acid.

results from the same site for wood (Kammer & Hagedorn, 2011; Jones *et al.*, 2011). The loss of pine wood C corresponds to annual to decadal turnover times, which is similar to that estimated by a 180 days laboratory incubation of the same wood in two Alfisols of different parents material (Santos *et al.*, 2012) and by a 1 year litter experiment using ^{13}C labeled twigs (Beech tree, 1–8 mm) in the same field study area (Kammer & Hagedorn, 2011; Jones *et al.*, 2011).

In the present field study, the mean difference of PyOM stocks over 10 months was approximately 1% of the amount added, but this difference was not significant so we did not estimate a turnover time of PyOM. Nevertheless, we can conclude that PyOM decomposed much slower than the plant biomass from which it was derived after pyrolysis. Several field and laboratory studies have found PyOM turnover times on centennial scale (Singh *et al.*, 2012). Our results provide *in situ* confirmation of the relative rates reported by previous laboratory studies. (Baldock & Smernik, 2002; Hilscher & Knicker, 2011; Santos *et al.*, 2012).

We recovered 3–4% of applied PyOM below the application depth (0–5 cm) suggesting downward vertical movement of PyOM. Leifeld *et al.* (2007) estimated migration rate of 630–1160 mm yr^{-1} in a peat soil with very low bulk density where 21–69% migrated below the incorporation depth in 95 years. Compared to the previously cited study, the soil here was denser, more clayey and so probably less favorable to organic matter transfer through the profile. Nevertheless, the amount of translocated PyOM to lower depth in the present case was higher than Major *et al.* (2009a) who observed 100 mm yr^{-1} migration rate in a sandy Oxisol under native savanna vegetation with only 0.45% of applied amount. Therefore, translocation of PyOM to lower depth in the present case could either be due to soil faunal mixing (Carcaillet, 2001; Topoliantz & Ponge, 2003; Eckmeier *et al.*, 2007a) or leaching. The downward vertical movement could either result in its accumulation at lower depths (Skjemstad *et al.*, 1999; Dai *et al.*, 2005; Rumpel *et al.*, 2006; Brodowski *et al.*, 2007) or loss from soil as dissolved organic carbon (DOC) (Hockaday *et al.*, 2007; Dittmar *et al.*, 2012; Ding *et al.*, 2013). On the other hand, we observed that wood C translocated to lower depths is double (4–8%) in comparison to PyOM C. Transport of wood-C into deeper soil horizons usually occurs as DOC (Yano *et al.*, 2005; Zalamea *et al.*, 2007). This could partly explain the larger amount of wood being translocated to deeper soil profile.

Quality of PyOM changes within one year in soil

After 10 months in soil, the total BPCA-C content of PyOM was unchanged, but the proportion of various BPCAs had changed. Aromatic condensation, measured as the relative contribution of B6CA to the total BPCA-C (Brodowski, 2005; Schneider *et al.*, 2010), decreased after 10 months, suggesting that PyOM partially degraded into smaller aromatic moieties. In contrast, Hammes *et al.* (2008), in a field study, observed a relative increase

Table 5 Microbial biomass 10 months after $^{13}C/^{15}N$-labeled wood or PyOM addition to soil mesocosms across all N treatments. Values correspond to the mean ($n = 6$) and values between brackets correspond to SE

	Soil microbial biomass C			Soil microbial biomass N			Excess ^{13}C in microbial biomass			C : N ratio of soil microbial biomass		
	$= (C_{fum}-C_{nfum})/0.45$, μg g^{-1} dry soil			$= (N_{fum}-N_{nfum})/0.68$, μg g^{-1} dry soil			μg g^{-1} dry soil					
Treatment	0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm	0–5 cm	5–10 cm	10–15 cm
Wood	699 (86)	581 (86)	427 (86)	70 (11)	87 (6)	51 (8)	0.22 (0.05)	0.23 (0.15)	0.02 (0.01)	9.8 (2.3)	6.9 (1.9)	8.8 (2.1)
PyOM	671 (77)	454 (77)	360 (86)	79 (27)	42 (8)	32 (7)	0.01 (0.00)	0.00 (0.00)	0.00 (0.00)	8.4 (4.2)	10.9 (3.3)	11.4 (3.7)
Control	661 (77)	496 (77)	417 (94)	78 (10)	63 (7)	12 (4)	–	–	–	8.5 (1.5)	7.9 (1.9)	31.8 (13.2)

in the B6CA molecular marker after 100 years *in situ* (no absolute change) suggesting relative preservation of condensed aromatic structures. Moreover, Schneider *et al.* (2011) found no change in the BPCAs pattern in a 100-year chronosequence. It is not clear yet if the changes in the relative contribution of various BPCAs could be linked directly to decomposition mechanism. Abiven *et al.* (2011) observed an increase in the B3CA at the expense of B5CA between a fresh and a 10 year aged PyOM while Brodowski (2005) also observed similar results in an incubation study on the decomposition of PyOM. The dominance of B3CA and B4CA indicates small aromatic cluster size (Schneider *et al.*, 2011) and indicates depolymerization of the highly condensed aromatic backbone of PyOM. These previous findings, together with our data, suggest that PyOM in soil degrades by the breaking of condensed aromatic structures into smaller clusters, at least within the first stages of degradation after the input of PyOM to the soil.

PyOM was physically associated with the soil mineral fraction after 10 months

About one third of the applied PyOM C was recovered in aggregates (i.e., oLF) plus dense fraction of soil within 1 year. Studies of the ambient distribution of PyOM in soil fractions, in other words, in soils collected from sites without intentional addition of PyOM also find significant portion of PyOM in these fractions (Glaser *et al.*, 2000; Brodowski *et al.*, 2006; Laird *et al.*, 2008; Liang *et al.*, 2008; Vasilyeva *et al.*, 2011). However, these studies do not report the temporal scale over which aggregation and/or organo-mineral interaction of PyOM with soil occurs, as they have no data for PyOM inputs to these soils. This study highlights, for the first time, that significant interaction between PyOM and the mineral phase of soil can occur *in situ* within a year. Glaser *et al.* (2000) posit that interaction of PyOM and soil mineral phases might stabilize PyOM by aggregation and organo-mineral associations. As one mechanism, oxidation of PyOM surface has been hypothesized to favor its interaction with the soil mineral phase (Brodowski *et al.*, 2006). It was, however, not possible to directly link oxidized forms of PyOM to a specific interaction with soil minerals that led to recovery of PyOM in the dense fraction. Moreover, the presence of PyOM in DF does not mean that all of it was stable organo-mineral associates; PyOM in the DF could also occur as an uncomplexed sand fraction.

PyOM accelerated the loss of native C from fLF

We observed positive priming in the free particulate native C pool in soil (i.e., in the fLF) by both PyOM and

wood, with significantly larger priming by PyOM than wood of native SOC in the fLF. However, there is no consistent effect of PyOM on native SOC across the literature. For example, Santos *et al.* (2012) did not observe priming in SOM using the same substrates but different soils in an incubation study, which indicated that the type of soil has an influence on priming effects rather than the organic substrate itself. In a recent study under controlled conditions, Stewart *et al.* (2013) observed an exponential relationship between initial SOC and cumulative soil C primed by PyOM addition with high negative priming at low soil C% and positive priming at high soil C%. Our study, for the first time, indicated which pool of SOM is affected due to priming by organic input. The native SOM associated to the minerals was not affected by the input while the SOM that was free or occluded in aggregates decreased significantly within few months.

One property supporting priming is PyOM's porous structure which is known to sorb organic substrates (Raveendran & Ganesh, 1998; Sudhakar & Dikshit, 1999; Accardi-Dey & Gschwend, 2002; Kwon & Pignatello, 2005; Chen *et al.*, 2008) and may offer favorable microsites for microorganisms and shelter them against soil faunal predators (Pietikäinen *et al.*, 2000). If so, the physical effects of PyOM amendments could increase microbial activity and lead to increased mineralization of readily decomposable substrates such as the fLF. However, we were not able to detect a change in microbial biomass (see No change in microbial biomass), and further research is needed to develop a predictive understanding of the temporal course of priming-type effects.

No change in microbial biomass

Pyrogenic organic matter addition had no effect on SMB-C 10 months after addition to the soil mesocosm. Bruun *et al.* (2008) observed a similar lack of effect in soil treated with PyOM (^{14}C-labeled roots of barley). On the contrary, Steinbeiss *et al.* (2009) observed a reduction in microbial biomass in soil to which charred glucose had been added to a forest soil in an incubation study after 4 months. Several studies observed increased microbial biomass and activity in soil (Steiner *et al.*, 2008; Kolb *et al.*, 2009; Bruun *et al.*, 2011) within days to few months after PyOM addition or higher SMB-C in PyOM rich soils compared to control or adjacent soils with no PyOM (Liang *et al.*, 2010). Our study was comparatively longer than the studies cited above (10 months vs. a couple of weeks) and therefore we cannot exclude the possibility that the microbial biomass could have increased in the first few weeks after PyOM addition and reverted to its initial value over time. Moreover, this study is the case of a single input of PyOM to the soil without a real wildfire and therefore its effect on the soil properties and consequently on microbial biomass is not similar to PyOM rich soils. In addition, the inconsistency in the response of microbial biomass to PyOM treatment in soil may be attributed to type of PyOM used that differed in the degree of condensation, intensity of pyrolysis and amount of precombustion material present in various studies (Zavalloni *et al.*, 2011). PyOM could have influenced the extraction efficiency but in our study it is not likely that it would have resulted in a major change, as the amount of PyOM used was small as compared to total SOC. Our results suggest that, under these conditions, the total microbial biomass is not durably affected by the substrate addition.

We observed a small amount of PyOM-C within the microbial biomass 10 months after organic inputs to soil, as did Bruun *et al.* (2008) and Zavalloni *et al.* (2011). Kuzyakov *et al.* (2009) and Kolb *et al.* (2009) observed a higher assimilation of PyOM (1.5–2.6% of initial PyOM input) by microbial biomass after 624 days of incubation compared to our 10 months field study. In all these studies, the amount of PyOM incorporated into the SMB was large enough to be detected clearly, and hence indicate that microbes can utilize PyOM as a C source.

Effect of N fertilization

The addition of N had little impact on the parameters considered in this study. Under the added N treatment, we observed a significant increase in the total C content at 0–5 cm depth in wood-amended soil, higher loss of wood-N, a higher transfer of wood derived C in the DF and a higher N content in the microbial biomass. However, these changes are limited and affect the C and N cycles only marginally. These results are in line with Santos *et al.* (2012). However, the effect of N addition is often seen in the longer term and these results need to be validated for longer periods.

Summary and environmental implications

The novel use of a dual isotope label for PyOM and wood in this field study provided insight into the dynamics of PyOM and wood C and N during the first year after its application to soil. The PyOM C was almost completely retained in the soil, although there was a small but significant change in the overall chemical structure of PyOM. In contrast, 48% of the initial amount of wood C was lost. Our results showed that PyOM persistence in soil need not solely be due to its chemical structure, as one third of the PyOM C was quickly incorporated into physically protected fractions

(i.e., oLF and DF). PyOM primed the loss of native soil C in the free light fraction, suggesting a significant loss of decadal C pool of SOM.

This study shows that PyOM seems to be a promising tool to stabilize C in soils. After 10 months, PyOM-C losses from soil were negligible; PyOM-C was hardly assimilated by microbes and seemed to interact substantially with the mineral phase of the soil, leading to a potential long-term stabilization of the C. However, this study also shows that the application of PyOM increased the priming of the easily decomposable organic matter of the soil and may offset its stabilization. While a lot of attention has been brought to the chemistry of the PyOM as main driver of its stability in the soil, our study rather highlights that its interactions with the mineral and organic phases of the soil may be quantitatively more relevant and more dynamic than its chemical structure, at least on the short term. This new information would advocate a better understanding of PyOM relationships to soil characteristics in future mechanistic investigations.

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References

Abbott DT, Crossley DA (1982) Woody litter decomposition following clear-cutting. *Ecology*, **63**, 35–42.

Abiven S, Andreoli R (2010) Charcoal does not change the decomposition rate of mixed litters in a mineral cambisol: a controlled conditions study. *Biology and Fertility of Soils*, **47**, 111–114.

Abiven S, Hengartner P, Schneider MPW, Singh N, Schmidt MWI (2011) Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. *Soil Biology & Biochemistry*, **43**, 1615–1617.

Accardi-Dey A, Gschwend PM (2002) Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. *Environmental Science & Technology*, **36**, 21–29.

Allison SD, Lebauer DS, Ofrecio MR, Reyes R, Ta AM, Tran TM (2009) Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. *Soil Biology & Biochemistry*, **41**, 293–302.

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally, altered Pinus resinosa (Red Pine) wood. *Organic Geochemistry*, **33**, 1093–1109.

Bebber DP, Watkinson SC, Boddy L, Darrah PR (2011) Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. *Oecologia*, **167**, 1177–1184.

Bernoux M, Cerri CC, Neill C, De Moraes JFL (1998) The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma*, **82**, 43–58.

Bird JA, Torn MS (2006) Fine roots vs. Needles: a comparison of C-13 and N-15 dynamics in a ponderosa pine forest soil. *Biogeochemistry*, **79**, 361–382.

Bird MI, Moyo C, Veenendaal EM, Lloyd J, Frost P (1999) Stability of elemental carbon in a savanna soil. *Global Biogeochemical Cycles*, **13**, 923–932.

Blagodatskaya E, Kuzyakov Y (2008) Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. *Biology and Fertility of Soils*, **45**, 115–131.

Bragazza L, Freeman C, Jones T *et al.* (2006) Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 19386–19389.

Brodowski S (2005) Origin, function, and reactivity of black carbon in the arable soil environment. PhD Thesis, Institut für Bodenkunde, Bonn.

Brodowski S, Amelung W, Haumaier L, Abetz C, Zech W (2005a) Morphological and chemical properties of black carbon in physical soil fractions as revealed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. *Geoderma*, **128**, 116–129.

Brodowski S, Rodionov A, Haumaier L, Glaser B, Amelung W (2005b) Revised black carbon assessment using benzene polycarboxylic acids. *Organic Geochemistry*, **36**, 1299–1310.

Brodowski S, John B, Flessa H, Amelung W (2006) Aggregate-occluded black carbon in soil. *European Journal of Soil Science*, **57**, 539–546.

Brodowski S, Amelung W, Haumaier L, Zech W (2007) Black carbon contribution to stable humus in German arable soils. *Geoderma*, **139**, 220–228.

Bruun S, Jensen ES, Jensen LS (2008) Microbial mineralization and assimilation of black carbon: dependency on degree of thermal alteration. *Organic Geochemistry*, **39**, 839–845.

Bruun EW, Muller-Stover D, Ambus P, Hauggaard-Nielsen H (2011) Application of biochar to soil and N2O emissions: potential effects of blending fast-pyrolysis biochar with anaerobically digested slurry. *European Journal of Soil Science*, **62**, 581–589.

Busse MD (1994) Downed bole-wood decomposition in lodgepole pine forests of central Oregon. *Soil Science Society of America Journal*, **58**, 221–227.

Carcaillet C (2001) Are Holocene wood-charcoal fragments stratified in alpine and subalpine soils? Evidence from the Alps based on AMS C-14 dates. *Holocene*, **11**, 231–242.

Cerli C, Celi L, Kalbitz K, Guggenberger G, Kaiser K (2012) Separation of light and heavy organic matter fractions in soil - Testing for proper density cut-off and dispersion level. *Geoderma*, **170**, 403–416.

Chambers JQ, Schimel JP, Nobre AD (2001) Respiration from coarse wood litter in central Amazon forests. *Biogeochemistry*, **52**, 115–131.

Chatterjee S, Santos F, Abiven S, Itin B, Stark RE, Bird JA (2012) Elucidating the chemical structure of pyrogenic organic matter by combining magnetic resonance, mid-infrared spectroscopy and mass spectrometry. *Organic Geochemistry*, **51**, 34–44.

Chen BL, Zhou DD, Zhu LZ (2008) Transitional adsorption and partition of nonpolar and polar aromatic contaminants by biochars of pine needles with different pyrolytic temperatures. *Environmental Science & Technology*, **42**, 5137–5143.

Cheng CH, Lehmann J (2009) Ageing of black carbon along a temperature gradient. *Chemosphere*, **75**, 1021–1027.

Cheng CH, Lehmann J, Thies JE, Burton SD, Engelhard MH (2006) Oxidation of black carbon by biotic and abiotic processes. *Organic Geochemistry*, **37**, 1477–1488.

Clark DB, Clark DA, Brown S, Oberbauer SF, Veldkamp E (2002) Stocks and flows of coarse woody debris across a tropical rain forest nutrient and topography gradient. *Forest Ecology and Management*, **164**, 237–248.

Crow SE, Lajtha K, Filley TR, Swanston CW, Bowden RD, Caldwell BA (2009) Sources of plant-derived carbon and stability of organic matter in soil: implications for global change. *Global Change Biology*, **15**, 2003–2019.

Dai X, Boutton TW, Glaser B, Ansley RJ, Zech W (2005) Black carbon in a temperate mixed-grass savanna. *Soil Biology & Biochemistry*, **37**, 1879–1881.

Dawson TE, Mambelli S, Plamboeck AH, Templer PH, Tu KP (2002) Stable isotopes in plant ecology. *Annual Review of Ecology and Systematics*, **33**, 507–559.

Deluca TH, Aplet GH (2008) Charcoal and carbon storage in forest soils of the Rocky Mountain West. *Frontiers in Ecology and the Environment*, **6**, 18–24.

Ding Y, Yamashita Y, Dodds WK, Jaffe R (2013) Dissolved black carbon in grassland streams: is there an effect of recent fire history? *Chemosphere*, **90**, 2557–2562.

Dittmar T, Paeng J, Gihring TM, Suryaputra IGNA, Huettel M (2012) Discharge of dissolved black carbon from a fire-affected intertidal system. *Limnology and Oceanography*, **57**, 1171–1181.

Durenkamp M, Luo Y, Brookes PC (2010) Impact of black carbon addition to soil on the determination of soil microbial biomass by fumigation extraction. *Soil Biology & Biochemistry*, **42**, 2026–2029.

Eckmeier E, Gerlach R, Skjemstad JO, Ehrmann O, Schmidt MWI (2007a) Minor changes in soil organic carbon and charcoal concentrations detected in a temperate deciduous forest a year after an experimental slash-and-burn. *Biogeosciences*, **4**, 377–383.

Eckmeier E, Rosch M, Ehrmann O, Schmidt MWI, Schier W, Gerlach R (2007b) Conversion of biomass to charcoal and the carbon mass balance from a slash-and-burn experiment in a temperate deciduous forest. *Holocene*, **17**, 539–542.

F.A.O.-U.N.E.S.C.O. (1998) *ISSS, ISRIC, FAO. World Reference Base for Soil Resources*. World Soil Resources Reports 84. FAO, Rome.

Fasth BG, Harmon ME, Sexton J, White P (2011) Decomposition of fine woody debris in a deciduous forest in North Carolina. *Journal of the Torrey Botanical Society*, **138**, 192–206.

Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic-Matter. *Biological Reviews of the Cambridge Philosophical Society*, **63**, 433–462.

Fontaine S, Mariotti A, Abbadie L (2003) The priming effect of organic matter: a question of microbial competition? *Soil Biology & Biochemistry*, **35**, 837–843.

Galloway JN, Townsend AR, Erisman JW *et al.* (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science*, **320**, 889–892.

Glaser B, Haumaier L, Guggenberger G, Zech W (1998) Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Organic Geochemistry*, **29**, 811–819.

Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W (2000) Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Organic Geochemistry*, **31**, 669–678.

Goldberg ED (1985) *Black Carbon in the Environment: Properties and Distribution*. John Wiley and Sons, New York.

Guo LB, Bek E, Gifford RM (2006) Woody debris in a 16-year old Pinus radiata plantation in Australia: mass, carbon and nitrogen stocks, and turnover. *Forest Ecology and Management*, **228**, 145–151.

Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry*, **35**, 823–830.

Hammes K, Smernik RJ, Skjemstad JO, Herzog A, Vogt UF, Schmidt MWI (2006) Synthesis and characterisation of laboratory-charred grass straw (Oryza saliva) and chestnut wood (Castanea sativa) as reference materials for black carbon quantification. *Organic Geochemistry*, **37**, 1629–1633.

Hammes K, Torn MS, Lapenas AG, Schmidt MWI (2008) Centennial black carbon turnover observed in a Russian steppe soil. *Biogeosciences*, **5**, 1339–1350.

Hilscher A, Knicker H (2011) Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. *Organic Geochemistry*, **42**, 42–54.

Hilscher A, Heister K, Siewert C, Knicker H (2009) Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. *Organic Geochemistry*, **40**, 332–342.

Hockaday WC, Grannas AM, Kim S, Hatcher PG (2006) Direct molecular evidence for the degradation and mobility of black carbon in soils from ultrahigh-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. *Organic Geochemistry*, **37**, 501–510.

Hockaday WC, Grannas AM, Kim S, Hatcher PG (2007) The transformation and mobility of charcoal in a fire-impacted watershed. *Geochimica Et Cosmochimica Acta*, **71**, 3432–3445.

Jackson ML (1958) *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs, NJ, USA.

Janssens IA, Dieleman W, Luyssaert S *et al.* (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience*, **3**, 315–322.

Jones DL, Murphy DV, Khalid M, Ahmad W, Edwards-Jones G, Deluca TH (2011) Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. *Soil Biology & Biochemistry*, **43**, 1723–1731.

Kammer A, Hagedorn F (2011) Mineralisation, leaching and stabilisation of C-13-labelled leaf and twig litter in a beech forest soil. *Biogeosciences*, **8**, 2195–2208.

Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar). *Environmental Science & Technology*, **44**, 1247–1253.

Kloeti P, Keller HM, Guecheva M (1989) Effects of forest canopy on throughfall precipitation chemistry. *Atmospheric Deposition, Proceedings of the Baltimore Symposium*. IAHS Publ, Wallingford, UK, **179**, 203–209.

Knicker H (2011) Pyrogenic organic matter in soil: its origin and occurrence, its chemistry and survival in soil environments. *Quaternary International*, **243**, 251–263.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. *Ecology*, **86**, 3252–3257.

Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of charcoal quantity on microbial biomass and activity in temperate soils. *Soil Science Society of America Journal*, **73**, 1173–1181.

Kuzyakov Y, Subbotina I, Chen HQ, Bogomolova I, Xu XL (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. *Soil Biology & Biochemistry*, **41**, 210–219.

Kwon S, Pignatello JJ (2005) Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): pseudo pore blockage by model lipid components and its implications for N-2-probed surface properties of natural sorbents. *Environmental Science & Technology*, **39**, 7932–7939.

Laird DA, Chappell MA, Martens DA, Wershaw RL, Thompson M (2008) Distinguishing black carbon from biogenic humic substances in soil clay fractions. *Geoderma*, **143**, 115–122.

Lehmann J, Gaunt J, Rondon M (2006) Bio-Char sequestration in terrestrial ecosystems- A review. *Mitigation and Adaptation Strategies for Global Change*, **11**, 403–427.

Lehmann J, Skjemstad J, Sohi S *et al.* (2008) Australian climate-carbon cycle feedback reduced by soil black carbon. *Nature Geoscience*, **1**, 832–835.

Leifeld J, Fenner S, Muller M (2007) Mobility of black carbon in drained peatland soils. *Biogeosciences*, **4**, 425–432.

Liang B, Lehmann J, Solomon D *et al.* (2008) Stability of biomass-derived black carbon in soils. *Geochimica Et Cosmochimica Acta*, **72**, 6069–6078.

Liang BQ, Lehmann J, Sohi SP *et al.* (2010) Black carbon affects the cycling of non-black carbon in soil. *Organic Geochemistry*, **41**, 206–213.

Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin FS (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature*, **431**, 440–443.

Major J, Lehmann J, Rondon M, Goodale C (2009a) Fate of soil-applied black carbon: downward migration, leaching and soil respiration. *Global Change Biology*, **16**, 1366–1379.

Major J, Steiner C, Downie A, Lehamann J (2009b) Biochar effects on nutrient leaching. In: *Biochar for Environmental Management Science and Technology*. (eds Lehman J, Joseph S), pp. 271–287. Earthscan, London.

Mccoll JG, Powers RF (1998) Decomposition of small diameter woody debris of red fir determined by nuclear magnetic resonance. *Communications in Soil Science and Plant Analysis*, **29**, 2691–2704.

Micks P, Downs MR, Magill AH, Nadelhoffer KJ, Aber JD (2004) Decomposing litter as a sink for N-15-enriched additions to an oak forest and a red pine plantation. *Forest Ecology and Management*, **196**, 71–87.

Moritz MA, Parisien M-A, Batllori E, Krawchuk MA, Dorn JV, Ganz DJ, Hayhole K (2012) Climate change and disruptions to global fire activity. *Ecosphere*, **3**, 1–22.

Murage EW, Voroney PR (2007) Modification of the original chloroform fumigation extraction technique to allow measurement of delta C-13 of soil microbial biomass carbon. *Soil Biology & Biochemistry*, **39**, 1724–1729.

Nguyen BT, Lehmann J, Kinyangi J, Smernik R, Riha SJ, Engelhard MH (2008) Long-term black carbon dynamics in cultivated soil. *Biogeochemistry*, **89**, 295–308.

Norby RJ (1998) Nitrogen deposition: a component of global change analyses. *New Phytologist*, **139**, 189–200.

Pietikäinen J, Kiikkila O, Fritze H (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos*, **89**, 231–242.

Raveendran K, Ganesh A (1998) Adsorption characteristics and pore-development of biomass-pyrolysis char. *Fuel*, **77**, 769–781.

Rovira P, Duguy B, Vallejo VR (2009) Black carbon in wildfire-affected shrubland Mediterranean soils. *Journal of Plant Nutrition and Soil Science*, **172**, 43–52.

Ruehr NK, Buchmann N (2010) Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration. *Tree Physiology*, **30**, 165–176.

Rumpel C, Alexis M, Chabbi A, Chaplot V, Rasse DP, Valentin C, Mariotti A (2006) Black carbon contribution to soil organic matter composition in tropical sloping land under slash and burn agriculture. *Geoderma*, **130**, 35–46.

Santos F, Torn MS, Bird JA (2012) Biological degradation of pyrogenic organic matter in temperate forest soils. *Soil Biology & Biochemistry*, **51**, 115–124.

Schmidt MWI, Noack AG (2000) Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. *Global Biogeochemical Cycles*, **14**, 777–793.

Schmidt MWI, Rumpel C, Kogel-Knabner I (1999a) Evaluation of an ultrasonic dispersion procedure to isolate primary organomineral complexes from soils. *European Journal of Soil Science*, **50**, 87–94.

Schmidt MWI, Skjemstad JO, Gehrt E, Kogel-Knabner I (1999b) Charred organic carbon in German chernozemic soils. *European Journal of Soil Science*, **50**, 351–365.

Schmidt MWI, Torn MS, Abiven S *et al*. (2011) Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49–56.

Schneider MPW, Hilf M, Vogt UF, Schmidt MWI (2010) The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 degrees C and 1000 degrees C. *Organic Geochemistry*, **41**, 1082–1088.

Schneider MPW, Lehmann J, Schmidt MWI (2011) Charcoal quality does not change over a century in a tropical agro-ecosystem. *Soil Biology & Biochemistry*, **43**, 1992–1994.

Shen SM, Pruden G, Jenkinson DS (1984) Mineralization and Immobilization of Nitrogen in Fumigated Soil and the Measurement of Microbial Biomass Nitrogen. *Soil Biology & Biochemistry*, **16**, 437–444.

Shinogi Y, Yoshida H, Koizumi T, Yamaok M, Saito T (2003) Basic characteristics of low-temperature carbon products from waste sludge. *Advances in Environmental Research*, **7**, 661–665.

Singh N, Abiven S, Torn MS, Schmidt MWI (2012) Fire-derived organic carbon in soil turns over on a centennial scale. *Biogeosciences*, **9**, 2847–2857.

Skjemstad JO, Taylor JA, Janik LJ, Marvanek SP (1999) Soil organic carbon dynamics under long-term sugarcane monoculture. *Australian Journal of Soil Research*, **37**, 151–164.

Skjemstad JO, Reicosky DC, Wilts AR, Mcgowan JA (2002) Charcoal carbon in US agricultural soils. *Soil Science Society of America Journal*, **66**, 1249–1255.

Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology & Biochemistry*, **41**, 1301–1310.

Steiner C, Das KC, Garcia M, Forster B, Zech W (2008) Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. *Pedobiologia*, **51**, 359–366.

Stewart C, Zheng JY, Botte J, Cotrufo MF (2013) Co-generated fast pyrolysis biochar mitigates greenhouse gas emissions and increases carbon sequestration in temperate soils. *Global Change Biology Bioenergy*, **5**, 153–164.

Sudhakar Y, Dikshit AK (1999) Kinetics of endosulfan sorption on to wood charcoal. *Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes*, **34**, 587–615.

Sulzman EW, Brant JB, Bowden RD, Lajtha K (2005) Contribution of aboveground litter, belowground litter, and rhizosphere respiration to total soil CO2 efflux in an old growth coniferous forest. *Biogeochemistry*, **73**, 231–256.

Topoliantz S, Ponge JF (2003) Burrowing activity of the geophagous earthworm Pontoscolex corethrurus (Oligochaeta: Glossoscolecidae) in the presence of charcoal. *Applied Soil Ecology*, **23**, 267–271.

Turunen J, Roulet NT, Moore TR, Richard PJH (2004) Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in Eastern Canada. *Global Biogeochemical Cycles*, **18**, GB3002, doi: 3010.1029/2003GB002154.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. *Soil Biology & Biochemistry*, **19**, 703–707.

Vasilyeva NA, Abiven S, Milanovskiy EY, Hilf M, Rizhkov OV, Schmidt MWI (2011) Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem. *Soil Biology & Biochemistry*, **43**, 1985–1988.

Von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *European Journal of Soil Science*, **57**, 426–445.

Wal A, Boer W, Smant W, Veen J (2007) Initial decay of woody fragments in soil is influenced by size, vertical position, nitrogen availability and soil origin. *Plant and Soil*, **301**, 189–201.

Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C (2004) Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications*, **14**, 1172–1177.

Westerling AL, Hidalgo HG, Cayan DR, Swetnam TW (2006) Warming and earlier spring increase western US forest wildfire activity. *Science*, **313**, 940–943.

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of soil microbial biomass C by fumigation extraction - an automated procedure. *Soil Biology & Biochemistry*, **22**, 1167–1169.

Yano Y, Lajtha K, Sollins P, Caldwell BA (2005) Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on Andic soils: effects of litter quality. *Ecosystems*, **8**, 286–300.

Yarnes C, Santos F, Singh N, Abiven S, Schmidt MWI, Bird JA (2011) Stable isotopic analysis of pyrogenic organic matter in soils by liquid chromatography-isotope-ratio mass spectrometry of benzene polycarboxylic acids. *Rapid Communications in Mass Spectrometry*, **25**, 3723–3731.

Zalamea M, Gonzalez G, Ping CL, Michaelson G (2007) Soil organic matter dynamics under decaying wood in a subtropical wet forest: effect of tree species and decay stage. *Plant and Soil*, **296**, 173–185.

Zavalloni C, Alberti G, Biasiol S, Delle Vedove G, Fornasier F, Liu J, Peressotti A (2011) Microbial mineralization of biochar and wheat straw mixture in soil: a short-term study. *Applied Soil Ecology*, **50**, 45–51.

Zell J, Kandler G, Hanewinkel M (2009) Predicting constant decay rates of coarse woody debris-A meta-analysis approach with a mixed model. *Ecological Modelling*, **220**, 904–912.

Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science & Technology*, **44**, 1295–1301.

Zimmerman AR, Gao B, Ahn MY (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology & Biochemistry*, **43**, 1169–1179.

Manuscript III

Microbial community structure at family rank changes in PyOM amended temperate forest soil after one year

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Abstract

The addition of pyrogenic organic matter (PyOM) to the soil has been proposed to store carbon on the long term. However, little is known about the effect of PyOM on the microbial community and activity in the soil. We compared the microbial activity and microbial community structure of a forest soil in a 1 year field experiment amended with PyOM, *Pinus ponderosa* wood (precursor biomass of PyOM) and no organic input (control soils) under ambient nitrogen (N) and increased N treatment. Out of the four enzyme activity tested (β-1, 4 glucosidase, BG; β-N-acetylglucosaminidase, NAG; β-cellobiohydrolase, CBH; and phenol oxidase), only the phenol oxidase activity was significantly higher in PyOM-amended soil under ambient N than for the wood and control. Pyrosequencing of amended and unamended soil samples revealed no shift in the microbial community structure at phyla level. Changes were found at family rank for both PyOM and wood-amended soil as compared to unamended control soils. PyOM-amended soil showed an increase in the relative abundance of family *Holophagaceae* (*Acidobacteria*), *Dermacoccaceae* and *Micrococcaceae* (*Actinobacteria*) and *Sphingobacteriaceae* (*Bacteriodetes*) while decrease in the family *Nannocystaceae* (*Deltaproteobacteria*). Added N had slightly higher influence in the shift of microbial community than organic input and affected more families in different phyla. This study shows that under our field conditions, little changes in the microbial community may be expected by the addition of PyOM and thus PyOM does not alter the soil microbial biodiversity.

Keywords: pyrogenic organic matter, enzyme activity, microbial community structure, pyrosequencing, increased nitrogen, soil organic matter

1. Introduction

Pyrogenic organic matter (PyOM), the solid residue produced through incomplete combustion of biomass, is an important constituent of soils and sediments (Forbes *et al.*, 2006, Kuhlbusch, 1998, Masiello, 2004, Preston & Schmidt, 2006). PyOM is considered as the most stable organic component in the soil to date (Schmidt et al., 2011), and has been proposed as a tool to store carbon in the soil on the long term (Fowles, 2007, Lehmann, 2007, Lehmann *et al.*, 2006). Moreover, PyOM has been proposed to improve soil fertility (Glaser *et al.*, 2002, Novak *et al.*, 2009, Spokas *et al.*, 2012) and recognized as an effective sorbent for organic compounds (Pignatello *et al.*, 2006, Sudhakar & Dikshit, 1999). Despite environmental significance of PyOM in soils, we have an elementary understanding of the microbial response that PyOM generates within soils. Since soil microbial community mediates 80-90% of the processes in soil (Nannipieri *et al.*, 2003), any change in their nature and function may be relevant to our understanding of PyOM soil persistence.

Most of the studies on Soil-PyOM-Microbe interaction focused on the effect on soil microbial biomass. Microbial biomass tends to be high in PyOM rich soil (for e.g. Amazonian dark earth, ADE) (Grossman *et al.*, 2010) and was observed to increase in PyOM-amended soil (Ameloot *et al.*, 2013, Kolb *et al.*, 2009, Steiner *et al.*, 2008, Wardle *et al.*, 1998, Zackrisson *et al.*, 1996). However, some studies also observed either a decrease or no change in the microbial biomass (Dempster *et al.*, 2012, Steinbeiss *et al.*, 2009). The variance in the observed effect could be due to difference in the experimental set-up, soil types, PyOM source and production conditions. Little information also exists on the enzymatic activity of soils amended with PyOM but they also vary across studies. In a series of short-term batch experiments, Bailey et al. (2011) observed a highly variable effect of PyOM on β-glucosidase, β-N-acetylglucosaminidase, lipase, and leucine aminopeptidase in different soils studied. For instance, β-glucosidase activity increased in the sandy loam soil but had no effect on silt loam and sandy soil amended with switchgrass-derived PyOM. In contrast, Paz-Ferreiro et al. (2012) observed a decrease in the β-glucosidase activity in sandy loam soil amended with sludge-derived PyOM. Therefore, PyOM could have variable effect on enzyme activity depending on soil

types and type of PyOM used. There are wide knowledge gaps with respect to PyOM and its effect on various soil enzyme activities.

Very little work has focused on PyOM effects on soil microbial community structure. Substantial microbial community changes in forest soils containing wildfire derived charcoal layers has been observed by Pietikäinen et al., (2000). However, it is difficult to link these changes directly to PyOM itself or the changes in soil properties associated to the fire event. For instance, burned forest soils when supplemented with PyOM showed no increase in the relative abundance of *Actinobacteria* as was observed for non-burned sites (Khodadad *et al.*, 2011). Santos et al., (2012) observed no change in soil microbial community compositions as defined by soil PLFA C biomarkers in two forest soil differing in parent material amended with PyOM in a 180 d incubation study. Studies have shown that microbial community changes were partly dependent on the type of biochar used (Steinbeiss *et al.*, 2009) or changes in the edaphic factors due to PyOM additions to soils, for example change in pH (Bååth *et al.*, 1995, Frostegard *et al.*, 1993).

More recently, both traditional isolation techniques and culture-independent molecular techniques (e.g. terminal restriction fragment length polymorphism, qPCR, 16S rRNA sequencing) were applied to examine soil microbial community structure in PyOM rich pre-Columbian soils (Terra Preta) and soil where modern pyrolysis-generated PyOM was applied. The studies on ADE soils showed a higher microbial biomass and abundance of culturable bacteria and fungi, but significantly lower respiratory activity and thus a higher metabolic efficiency (Jesus *et al.*, 2009, Liang *et al.*, 2008, O'Neill *et al.*, 2009). Kim et al. (2007) observed that the bacterial composition of ADE were similar to forest soil in the vicinity but ADE supported greater species richness. On the other hand, studies on PyOM amended soil showed changes in the microbial community structure. Khodadad et al. (2011) in an incubation study using both burned and unburned soil observed an increase in the relative abundance of bacteria within the phyla *Actinobacteria* and *Gemmatimonadetes* when PyOM was added. Moreover, Kolton et al. (Kolton *et al.*,

2011) observed that the relative abundance of phylum *Bacteriodetes* increased and *Proteobacteria* decreased in the root associated bacteria in PyOM-amended soil in a pot experiment.

Up to now, the effect of PyOM on soil microbial activity and structure were studied either in incubations or field-based experiments where the PyOM addition was not controlled. An important limitation of most of the incubation studies is that they are closed systems, which limits microbial transport, and are short-term (few weeks to months) to capture any ecological adaptation and diversification. Therefore, the relevance of these controlled studies to ecosystem-scale dynamics requires further investigation in situ at a longer time scale. Field studies could provide more realistic information. However, the existing studies are either strongly influenced by event like fires or are based on soils that received PyOM amendment centuries ago. Therefore, it is difficult to single out the effect of PyOM input from other changes occurring in the soil due to fire events. Moreover, the initial effect of PyOM additions to the soil in ADE remains unknown. These limits make the existing data difficult to extrapolate to modern inputs of PyOM to soils, particularly in the frame of its use in agriculture.

Nitrogen (N) deposition to soil is also an important factor impacting terrestrial ecosystems. N inputs have been shown to induce changes in the microbial communities, but the direction of these changes is not that clear. Microbial taxa associated with specific components of the soil N cycle (for example, nitrifiers) often change in relative abundance when soils are amended with N (Fierer *et al.*, 2012) However, significant change in microbial community occurred at the highest levels of N addition with increase in *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*, and lower relative abundances of *Acidobacteria* (Fierer *et al.*, 2012). In some cases, microbial diversity has been shown to decrease with N additions (Campbell *et al.*, 2010). Previous studies (Singh et al., submitted; Maestrini et al., submitted) have proposed that N addition to soil might decrease the decomposition of PyOM.

However, the mechanisms behind these changes are not clear. In particular, the role of microbial communities is not known.

In this present study, the main objectives were to (1) evaluate changes in microbial activity due to PyOM and N addition to soils using enzyme assay; (2) examine the overall taxonomic changes in bacterial community structure due to PyOM and its precursor biomass (wood) amendments via pyrosequencing of 16S rRNA gene under field conditions. To achieve these objectives, we studied temperate forest soils where pine-wood and pine-wood-derived PyOM has been decomposing for 10 months under ambient and increased N treatments.

2. Materials and methods

2.1 Study site and sampling

The study site was located in the wind-thrown area in a mixed beech temperate forest on the south facing side of Lägeren mountain (easternmost part of the Jura mountain range) at 680 m above sea level, situated 20 km northwest of Zurich (47° 28' 40.8" N, 8° 21' 55.2" E), Switzerland. The soil was cambisol (F.A.O. 1998). Details on soil, site characteristics and experimental set-up had been described previously (Singh et al., submitted). Briefly, experimental set up included 20 cm long and 10 cm diameter mesocosms (polyethylene tubes), which were inserted into the soil up to a depth of 15 cm from the surface. The study had three organic input (wood, PyOM and no input as control) and two levels of nitrogen. We added *Pinus ponderosa* wood at a rate of 189 g C m^{-2}. PyOM was obtained by charring the labeled wood at 450ºC for 5 hours under N_2 flux according to Hammes et al (2006) and added at a rate of 397 g C m^{-2}. We also had two treatment levels of nitrogen (20 kg N. $yr^{-1}.ha^{-1}$ as ambient N =N0 and 80 (+60) kg N. $yr^{-1}.ha^{-1}$ as added N = N+). The chemical characteristics of both wood and PyOM have been summarized in Santos et al., (2012) and Chatterjee et al., (2012). In March 2010 after the snow melted, 11.4 mg of $NH_4^+NO_3^-$ dissolved in 10 ml of water were added monthly (equivalent to 60 kg N

$ha^{-1}\,yr^{-1}$) to increased N (N+) treatment mesocosms, while equivalent amount of distilled water was added to ambient N (N0) treatment mesocosms.

We sampled the intact mesocosms (n=18) ca. 10 months after the organic input addition. After sampling, soil samples from each mesocosm (n =18) were divided into 3 depth profiles (0-5cm, 5-10cm and 10-15 cm). Each soil sample was homogenized and roots and bigger plant parts were manually removed. The soil were archived at -80ºC until analyses. For this study we used soils from top 0-5 cm for each treatment.

2.2 Soil enzyme assay

Four enzymes were selected for this study: β-1, 4 glucosidase (BG), β-N-acetylglucosaminidase (NAG), β-cellobiohydrolase (CBH) and phenol oxidase. These enzymes were selected because they are key hydrolytic and oxidative soil enzymes responsible for degradation of glucose (BG), cellulose (CBH), chitin(NAG) and other phenolic compounds (phenol oxidase). Fluorescence-based soil assays for BG, NAG and CBH were based on protocols using the following respective substrates: 4-methylumbelliferyl β-d-glucopyranoside (Sigma, M3633), 4-methylumbelliferyl-N-acetyl-β-d-glucosaminide (Sigma, M2133) and 4-Methylumbelliferyl β-D-cellobioside (Sigma, M6018). Colorimetric soil assays were conducted for Phenol oxidase using the substrates 3,4-Dihydroxy-L-phenylalanine (Sigma, D9628). For the fluorescence-based assays, standard curves were developed and the enzyme activities were calculated against the appropriate curve.

2.3 DNA extraction from soil

The DNA was extracted from approximately 2 g (wet weight) soil following previously published procedure (DeAngelis *et al.*, 2011), with few modifications. Briefly, soil was added to 2 ml CTAB (hexadecyltrimethylammonium bromide) extraction buffer (1 part 10% CTAB in 1M NaCl and 1 part 0.5M phosphate buffer, pH 7.5-8.0). We also added 200 µl 0.1M aluminium ammonium sulfate and 2ml phenol: chloroform: iso-amyl alcohol (25:24:1) to soil and extraction buffer mixture.

It was followed by bead-beating this mixture by adding 0.5 g 3-4 mm glass bead and 1 g garnet (Megakit), and shaken in a Fastprep instrument (Savant) at 5.5 m/s for 30 s. The extracts were purified with chloroform, followed by centrifuging at 16k *g* for 5 minutes at 4˚C to collect the aqueous phase. DNA was precipitated by adding 1 µl linear acrylamide and 2 volumes of PEG solution (30% polyethylene glycol in 1.6 M NaCl) to the extracted aqueous phase and incubated at room temperature overnight. The DNA pellet was obtained by centrifuging PEG solution at 16000 *g*, which was washed with 800 µl ice-cold 70% ethanol. Ethanol was removed and DNA pellet was resuspended in 50 µl diethyl pyrocarbonate (DEPC) water and purified using Allprep kit (Qiagen Valencia CA).

2.4 Polymerase Chain Reactions (PCR)

PCR is an enzymatic process that rapidly amplifies specific DNA sequences (Saiki et al., 1985). The bacterial 16S rRNA gene was amplified by PCR from soil DNA using universal bacterial primers 907R (5' CCGTCAATTCCTTTRAGTTT) and 515F (5' GTGCCAGCMGCCGCGGTAA) and Takara Bio Inc. Hot start Ex-Taq polymerase. The PCR thermocycler (GeneAmp PCR system 9600, Perkin-Elmer) was set as 98˚C for 30 s for denaturation, amplification (25 cycles) at 98˚C for 30 s, 66˚C for 30 s and 72˚C for 1 minute followed by extension cycle at 72ºC for 10 minutes. After the PCR, the replicates were combined and proceeded to concentration and purification steps.

2.5 Purification and concentration of PCR product

We added SPRI (Solid Phase reversible immobilization) beads (Agencourt AMPure XP, Beckman genomics, Cat A63880) to combined PCR products and mixed thoroughly by pipette mixing (Deangelis *et al.*, 1995). The mixed samples were incubated for 5 minutes for maximum recovery. This step binds PCR product ≥150bp to the magnetic beads. The tubes were placed in a magnetic plate for 2 minutes to separate beads from the solution. The supernatant was removed and 120 µl of fresh 75% ethanol was added and incubated for 1 minute. Ethanol was pipetted off and excess ethanol was allowed to evaporate. The tubes were removed from the

plate and 30µl of elution buffer was added. The tubes were placed back on the plate for 1 minute to separate the beads from the solution. The eluant was transferred to new tube, which contained the purified PCR products. The amplicon pools were quantified using Bioanalyzer and 50 ng of each sample were pooled in a 1.5 ml tube. The next step goal was to concentrate the amplicon pools. For this, 1/10 volume of sodium acetate and 0.8-1volume isopropanol were added and mixed by pipetting. The mixture was incubated at -80˚C for 15 minutes and centrifuged at 4˚C for 20 minutes. The supernatant was removed and pellet was washed with 1 volume of 70% ethanol. The pellet was air dried and resuspended in 20 µl TE buffer (10mM Tris-Acetate pH 8.0, 1mM EDTA).

2.6 Sequencing and sequence refinement

Equal amounts of purified PCR products were combined in a single tube and sent to UC Berkeley DNA Sequencing Facility to be run on a 454 GS-FLX Titanium™ (*machine name*). Reads between 200 and 1000 bp were used for downstream analysis in the Quantitative Insights Into Microbial Ecology (QIIME) pipeline (Caporaso *et al.*, 2010b). All sequences with a mean quality score below 25 and homopolymer run of 6 or more were removed. Chimeras were detected using USEARCH (www.drive5.com/usearch) and removed. Sequences were clustered at 97% pairwise identity using the UCLUST (Edgar, 2010) reference-based OTU picking method, where the reference dataset was the GreenGenes 99% (GG99_12_10, Werner et al. 2012) data set. A representative sequence from each OTU was aligned to the GG99 data set using PyNAST (Caporaso et al., 2010b). The concatenated alignment of OTUs was filtered to remove gaps and hypervariable regions using the GreenGenes Lane mask (DeSantis et al. 2006). A phylogenetic tree was constructed from the filtered alignment using FastTree (Price et al., 2010). Taxonomic assignments were done with the naïve Bayesian algorithm (Wang et al., 2007) developed for the RDP classifier (Cole et al., 2009) using the GG99 taxonomy data set as training (Werner et al. 2012). Phylogenetic β-diversity was quantified

with a weighted Unifrac distance matrix, which was constructed at a depth of coverage of 3316 sequences (normalized coverage).

2.7 Statistical analysis

We applied analysis of variance (ANOVA) to evaluate the effect of different inputs and two levels of nitrogen on each enzyme activity and, in cases of significant F statistics, the Tukey's minimum significant difference test was used to compare the mean values. Statistical significance was determined at $p<0.05$ unless otherwise stated. Pairwise UniFrac distances calculated for each soil samples were visualized using non-metric multidimensional scaling (NMDS) using R software.

3. Results and Discussion

3.2 Enzyme activity

There was no significant difference in enzyme activity for β-1, 4 glucosidase (BG), β-N-acetylglucosaminidase (NAG) and β-cellobiohydrolase (CBH) due to wood or PyOM addition (Fig. 1). The activity of BG, CBH and NAH tends to increase under added N for both PyOM and wood amended soil (not significant, except for BG with wood treatment). However, added N decreased the activity of the hydrolytic C degrading enzyme (BG and CBH) for unamended control soil.

In comparison to the three previous enzymes, phenol oxidase activity presented more contrasted results: the activity in PyOM amended soil under ambient N activity was significantly higher ($p<0.05$) than for the control and the wood. Under added N treatment, the differences tend to level off and were no more significant. Carreiro et al., (2000) also observed decline in phenol oxidase activity due to chronic N addition. The reduced activity of phenol oxidase under added N was not observed for wood amended or control soils.

These results partially confirm previous findings on enzymatic activity induced by PyOM. Unlike previous study that observed significant change in BG activity (Bailey

et al., 2011, Paz-Ferreiro *et al.*, 2012) due to PyOM amendment, we observed no significant difference than the control soil. The variability could be attributed to difference due to both soil type as well as PyOM used in our studies and previously reported studies. Since the inputs of organic matter took place 10 months before the sampling, the little changes observed could correspond to a return to the initial enzymatic activity. Kourtev et al (2002) observed a return to initial enzymatic activity level, one year after the input of forest litter, for four types of enzymes (including β-1, 4 glucosidase). This would indicate that the main decomposition phase might be over for the wood treatment. Regarding the PyOM treatment, the significantly higher enzymatic activity to phenol oxidase may indicate that the decomposition is still going on.

Overall, we observe a very limited effect of nitrogen on the enzymatic activity. Many studies observed effects of nitrogen on the enzymatic activity (Saiya-Cork *et al.*, 2002). This does not seem to be the case in our study. However, the duration of our study might be too short to observe these changes.

3.3 Effect of added organic inputs and nitrogen on microbial community structure

Pyrosequencing yielded 88080 individual sequences, comprising 140 unique phylotypes (families) and an average of 4325 sequences and 80 unique phylotypes per sample. The soil in the present study was on average dominated by phyla *Proteobacteria* (40%, with *Alphaproteobacteria* = 22%, *Betaproteobacteria* = 8%, *Gammaproteobacteria* = 6% and *Deltaproteobacteria* = 4%), *Actinobacteria* (28%), *Acidobacteria* (9%), *Plantomycetes* (6%) and *Bacteriodete* (5%), (Fig. 2). Other less dominant phyla that were identified in soils across all treatments was *Gemmatimonadetes* (2%), *Firmicutes* (0.9%), *Chloroflexi* (0.06%), *Verrucomicrobia* (0.2%), and *WS3* (0.2%). Similar relative abundances of bacterial phyla and proteobacterial classes were observed in other studies in forest soils (Janssen, 2006, Nacke *et al.*, 2011), with the notable exception of the phylum *Actinobacteria* that was the most dominant taxon in our study. *Actinobacteria* play significant role in the

degradation of lignocelluloses and have been shown the potential to degrade many other polymers occurring in soil and litter, including hemicelluloses, pectin, keratin, and chitin (Goodfellow & Williams, 1983).

At the phylum level, there was no significant difference in the relative abundance of bacteria either due to organic input or nitrogen treatment. In contrast to the incubation study using burned and unburned soil by Khodadad et al. (2011), we observed no shift in community structure at a higher taxonomic classification. Under ambient N treatment, *Actinobacteria* tend to decrease and *Bacteriodete* and *Proteobacteria* tend to increase in PyOM amended soil as compared to control soils. We observed an opposite trend for the wood amended soil but none of the changes are significant.

We performed a PCA of relative abundances of major phyla for different treatments to elucidate major community distribution pattern (Fig. 3). In the score plot of PCA, the soils under added N treatment formed a more coherent cluster together the first principal component (PC 1) while there was no pattern for organic input treatment. However, for all treatments, the three replicates do not plot close to each other. That would indicate that the field heterogeneity is larger than the effect of the different treatments. Moreover, visualization of the pairwise weighted UniFrac distances on nonmetric multidimensional scaling plots indicates that significant variability exist within and across the treatments (Fig. 4). Soils with the same N and substrate treatment do not necessarily harbor similar bacterial communities, as the variability between treatments exceeded the variability within a given input treatment.

At the class level, the relative abundance of *Gammaproteobacteria* significantly decreased in wood-amended soil compared to PyOM treatment under both levels of nitrogen and control under ambient N (Supplementary material). Moreover, relative abundance of *Holophage* (*Acidobacteria*) and *Thermomicrobia* (*Chloroflexi*) decreased significantly for both wood and PyOM amended soil under ambient N compared to unamended-control soils. The effect at class rank was more visible for

the N addition treatment. Added N resulted in the increase in the relative abundance of *Chloracidobacteria* (*Acidobacteria*) and *Gammaproteobacteria* (*Proteobacteria*) in both wood and PyOM amended soil. Wood-amended soil with added N also showed increase in the relative abundances of *SM1B09* (*Chlorobi*), *Thermomicrobia* (*Chloroflexi*), *Betaproteobacteria* (*Proteobacteria*) and *Opitutae* (*Verrucomicrobia*). Added N also resulted in the decrease of *Spartobacteria* (*Verrucomicrobia*) in PyOM-amended soil.

At family level, significant differences were found in the phyla *Actinobacteria* and class *Betaproteobacteria*. Wood and PyOM-amended soil under ambient N showed increase in the relative abundance for the family *Holophagaceae* (*Acidobacteria*) and decrease in *Nannocystaceae* (*Deltaproteobacteria*) and family of phylum *Elusimicrobia* ($p \leq 0.05$) with respect to control soils. In the PyOM-amended soil, we observed significant increase in the relative abundance of family *Dermacoccaceae* and *Micrococcaceae* (*Actinobacteria*) under ambient N and *Sphingobacteriaceae* (*Bacteriodetes*) under added N compared to control-unamended soil. In the wood-amended soil under added N treatment, we also observed a significant increase in the relative abundance of family *Kineosporiaceae*, *Streptosporangiaceae*, *Alcaligenaceae* and unidentified family of order *Stramenopiles* (*Cyanobacteria*) and order *CTD005-82B-02* (*Deltaproteobacteria*). Under ambient N, wood amended soil showed a decrease in the relative abundance of family *Microbacteriaceae* (*Actinobacteria*) as compared to both PyOM-amended and unamended-control soil.

Effect of added N was more pronounced at family rank and affected relative abundances of several families across organic input amendments. In wood-amended soil, added N resulted in significant increase in the relative abundance of family *Nakamurellaceae* (*Actinobacteria)*, *Clostridiaceae* (*Fermicutes)*, *Comamonadaceae* (*Betaproteobacteria* class*)*, *Bacteriovoracaceae* (*Deltaproteobacteria*) and *Opitutaceae* (*Verrucomicrobia*) while decrease in family *Frankiaceae*, *Streptomycetaceae* (*Actinobacteria*) and *Hyphomicrobiaceae* (*Alphaproteobacteria* class). Effect of added N was also observed in PyOM-amended soil with the increase

in the relative abundance in family of class *Chloracidobacteria* (*Acidobacteria*) and decrease in *Dermacoccaceae* and *Micrococcaceae* (*Actinobacteria*), and unidentified family of phylum *Gemmatimonadetes*. Control-unamended soil showed significant increase in the relative abundance of family of class *Acidobacteria-5* and order *Burkholderiales* (*Betaproteobacteria*) while a decrease in the family *Holophagaceae* (*Acidobacteria*) and *Nannocystaceae* (*Deltaproteobacteria*) due to added N. A similar result was observed in two long-term N fertilization experiment on a grassland and an agricultural field where relative abundance of many of the dominant actinobacterial and proteobacterial groups, identified as copiotrophic taxa (those taxa that thrive in conditions of elevated C availability and exhibit relatively rapid growth rates, increased across the gradient (Fierer *et al.*, 2012).

The genera grouped under family *Nakamurellaceae* play an important role in the degradation of plant material (celluloses, hemicelluloses, etc.) and therefore their increase in wood-amended soil under added N indicates growth of species of this family with the availability of nutrients. PyOM-amendment in a field experiment was observed to potentially enhance the growth of organisms involved in N cycling (Anderson *et al.*, 2011). *Hyphomicrobiaceae* that could utilize N_2, NO_3^- or NH_3 soil treatment also decreased in the relative abundance in wood-amended soil under added N treatment. Added N, therefore, could decrease the growth of organisms involved in N cycling. Family of the order *Chloracidobacteria* is photosynthetic bacteria and therefore added N could stimulate its growth and increase its relative abundance.

Overall, the N addition induces changes at a higher hierarchical level (class) than the organic input (family) and also affects more families. Under field conditions, nitrogen addition has been shown to have effect at the phyla level when applied at high level, i.e. above 250 kg N ha^{-1} yr^{-1} (Fierer *et al.*, 2012). Our results corresponding to the N addition confirm these results: since our N addition level is relatively low, the changes are only visible at a lower level of the microbial

community structure. This would also indicate that, under our study conditions, very little changes are induced by the PyOM and wood inputs.

3.5 Network analysis

We performed network analysis of taxon co-occurrence patterns to get new insight into change in the structure of complex microbial communities due to organic input and N treatments (Supplementary data). The network analysis revealed that with added N treatment correlation between the relative abundance for family *Solibacteraceae, Comamonadaceae* and *Xanthomonadaceae* and the relative abundance of other families was lost. Significant correlation in the relative abundance appeared with other families of phylum *Acidobacteria*, *Actinobacteria* and *Bacteroidetes* with increased N treatment. This indicates that the global bacterial community structure was only weakly affected by the N addition and organic inputs.

3.6 Correlation between edaphic factors and bacterial communities

Soil properties such as pH value or soil texture are important drivers of bacterial community structure (Rousk *et al.*, 2010). PyOM have been considered to change edaphic properties and therefore indirectly could change the bacterial community structure (Sohi *et al.*, 2010). We used Spearman's rank correlation to identify relationships between relative abundance of major phyla and soil properties (Table 2). The relative abundance of different bacterial group responded to soil C and N content. At phylum level, relative abundance of *Bacteriodete* increased with increase in total C ($p < 0.01$) and N ($p < 0.05$) content of soil. We also found strong positive correlation of total C and N of soil with relative abundance for class *Gammaproteobacteria* ($p < 0.05$). The relative abundance of bacterial groups also responded to pH. We observed that the relative abundance of class *Deltaproteobacteria* increased while *Alphaproteobacteria* decreased with increasing pH. Nacke et al., (2011) observed a similar trend for *Alphaproteobacteria* in German forest and grassland soils.

In our study, the pH, CEC, C and N values did not correlate with the organic matter amendments or the N addition (Singh et al. submitted). In particular, the pH of the PyOM was 7.4, and did not affect significantly the pH of the soil (5.9). Due to the relatively low input of wood and PyOM, the total carbon content was also not significantly changed. So, the correlations we observed above are rather related to the soil heterogeneity than the treatments. However, it may indicate that if the amendments could change these soil properties, larger changes in the microbial structures may occur. This would advocate for an indirect effect of PyOM and other amendment of microbial communities, via a change in the chemical properties of the soils.

4 Conclusion

In this study, we investigated the microbial activity and the microbial community structure of soils, in the field, 10 months after the addition of wood and PyOM and under ambient or increased N addition. The main conclusions are the following:

- We observed no changes in the enzymatic activity of these soils, except an increase of phenol oxidase due to PyOM inputs
- The changes in the microbial community are minimal, and mainly due to the addition of N. The changes due to PyOM addition are detectable only at the family level.

Our study suggests that, under field conditions, the microbial populations may not be strongly affected by PyOM addition, and so the effect of PyOM on the soil microbial diversity may be marginal. The modifications observed in soils like Terra preta might occur on the longer term, and may be rather due to changes in the soil properties.

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References

Ameloot, N., Neve, S., Jegajeevagan, K., Yildiz, G., Buchan, D., Funkuin, Y.N., Prins, W., Bouckaert, L., Sleutel, S., 2013. Short-term CO2 and N2O emissions and microbial properties of biochar amended sandy loam soils. Soil Biology & Biochemistry 57, 401-410.

Anderson, C.R., Condron, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., Sherlock, R.R., 2011. Biochar induced soil microbial community change: Implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia 54, 309-320.

Bååth, E., Frostegard, A., Pennanen, T., Fritze, H., 1995. Microbial Community Structure and pH Response in Relation to Soil Organic-Matter Quality in Wood-Ash Fertilized, Clear-Cut or Burned Coniferous Forest Soils. Soil Biology & Biochemistry 27, 229-240.

Bailey, V.L., Fansler, S.J., Smith, J.L., Bolton, H., 2011. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biology & Biochemistry 43, 296-301.

Campbell, B.J., Polson, S.W., Hanson, T.E., Mack, M.C., Schuur, E.A.G., 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environmental Microbiology 12, 1842-1854.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F.D., Costello, E.K., Fierer, N., Pena, A.G., Goodrich, J.K., Gordon, J.I., Huttley, G.A., Kelley, S.T., Knights, D., Koenig, J.E., Ley, R.E., Lozupone, C.A., McDonald, D., Muegge, B.D., Pirrung, M., Reeder, J., Sevinsky, J.R., Tumbaugh, P.J., Walters, W.A., Widmann, J., Yatsunenko, T., Zaneveld, J., Knight, R., 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7, 335-336.

Carreiro, M.M., Sinsabaugh, R.L., Repert, D.A., Parkhurst, D.F., 2000. Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81, 2359-2365.

Chatterjee, S., Santos, F., Abiven, S., Itin, B., Stark, R.E., Bird, J.A., 2012. Elucidating the chemical structure of pyrogenic organic matter by combining magnetic resonance, mid-infrared spectroscopy and mass spectrometry. Organic Geochemistry 51, 34-44.
DeAngelis, K.M., Wu, C.H., Beller, H.R., Brodie, E.L., Chakraborty, R., DeSantis, T.Z., Fortney, J.L., Hazen, T.C., Osman, S.R., Singer, M.E., Tom, L.M., Andersen, G.L., 2011. PCR Amplification-Independent Methods for Detection of Microbial Communities by the High-Density Microarray PhyloChip. Applied and Environmental Microbiology 77, 6313-6322.
Deangelis, M.M., Wang, D.G., Hawkins, T.L., 1995. Solid-phase reversible immobilization for the isolation of PCR products. Nucleic Acids Research 23, 4742-4743.
Dempster, D.N., Gleeson, D.B., Solaiman, Z.M., Jones, D.L., Murphy, D.V., 2012. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. Plant and Soil 354, 311-324.
F.A.O.-U.N.E.S.C.O., 1998. ISSS, ISRIC, FAO. World Reference Base for Soil Resources. FAO, World Soil Resources Reports 84, Rome.
Fierer, N., Lauber, C.L., Ramirez, K.S., Zaneveld, J., Bradford, M.A., Knight, R., 2012. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. Isme Journal 6, 1007-1017.
Forbes, M.S., Raison, R.J., Skjemstad, J.O., 2006. Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. Science of the Total Environment 370, 190-206.
Fowles, M., 2007. Black carbon sequestration as an alternative to bioenergy. Biomass & Bioenergy 31, 426-432.
Frostegard, A., Baath, E., Tunlid, A., 1993. Shifts in the Structure of Soil Microbial Communities in Limed Forests as Revealed by Phospholipid Fatty-Acid Analysis. Soil Biology & Biochemistry 25, 723-730.
Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal - a review. Biology and Fertility of Soils 35, 219-230.
Goodfellow, M., Williams, S.T., 1983. Ecology of Actinomycetes. Annual Review of Microbiology 37, 189-216.
Grossman, J.M., O'Neill, B.E., Tsai, S.M., Liang, B.Q., Neves, E., Lehmann, J., Thies, J.E., 2010. Amazonian Anthrosols Support Similar Microbial Communities that Differ Distinctly from Those Extant in Adjacent, Unmodified Soils of the Same Mineralogy. Microbial Ecology 60, 192-205.
Hammes, K., Smernik, R.J., Skjemstad, J.O., Herzog, A., Vogt, U.F., Schmidt, M.W.I., 2006. Synthesis and characterisation of laboratory-charred grass straw (Oryza saliva) and chestnut wood (Castanea sativa) as reference materials for black carbon quantification. Organic Geochemistry 37, 1629-1633.
Janssen, P.H., 2006. Identifying the dominant soil bacterial taxa in libraries of 16S rRNA and 16S rRNA genes. Applied and Environmental Microbiology 72, 1719-1728.
Jesus, E.d.C., Marsh, T.L., Tiedje, J.M., Moreira, F.M.d.S., 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils Isme Journal 3, 1222-1222.

Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., Foster, J.S., 2011. Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biology & Biochemistry 43, 385-392.
Kim, J.S., Sparovek, G., Longo, R.M., De Melo, W.J., Crowley, D., 2007. Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. Soil Biology & Biochemistry 39, 684-690.
Kolb, S.E., Fermanich, K.J., Dornbush, M.E., 2009. Effect of Charcoal Quantity on Microbial Biomass and Activity in Temperate Soils. Soil Science Society of America Journal 73, 1173-1181.
Kolton, M., Harel, Y.M., Pasternak, Z., Graber, E.R., Elad, Y., Cytryn, E., 2011. Impact of Biochar Application to Soil on the Root-Associated Bacterial Community Structure of Fully Developed Greenhouse Pepper Plants. Applied and Environmental Microbiology 77, 4924-4930.
Kourtev, P.S., Ehrenfeld, J.G., Huang, W.Z., 2002. Enzyme activities during litter decomposition of two exotic and two native plant species in hardwood forests of New Jersey. Soil Biology & Biochemistry 34, 1207-1218.
Kuhlbusch, T.A.J., 1998. Black carbon and the carbon cycle. Science 280, 1903-1904.
Lehmann, J., 2007. Bio-energy in the black. Frontiers in Ecology and the Environment 5, 381-387.
Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-Char sequestration in terrestrial ecosystems- A review. Mitigation and Adaptation Strategies for Global Change 11, 403-427.
Liang, B., Lehmann, J., Solomon, D., Sohi, S., Thies, J.E., Skjemstad, J.O., Luizao, F.J., Engelhard, M.H., Neves, E.G., Wirick, S., 2008. Stability of biomass-derived black carbon in soils. Geochimica Et Cosmochimica Acta 72, 6069-6078.
Masiello, C.A., 2004. New directions in black carbon organic geochemistry. Marine Chemistry 92, 201-213.
Nacke, H., Thurmer, A., Wollherr, A., Will, C., Hodac, L., Herold, N., Schoning, I., Schrumpf, M., Daniel, R., 2011. Pyrosequencing-Based Assessment of Bacterial Community Structure Along Different Management Types in German Forest and Grassland Soils. Plos One 6.
Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003. Microbial diversity and soil functions. European Journal of Soil Science 54, 655-670.
Novak, J.M., Busscher, W.J., Laird, D.L., Ahmedna, M., Watts, D.W., Niandou, M.A.S., 2009. Impact of biochar amendment on fertility of a southeastern coastal plain soil. Soil Science 174, 105-112.
O'Neill, B., Grossman, J., Tsai, M.T., Gomes, J.E., Lehmann, J., Peterson, J., Neves, E., Thies, J.E., 2009. Bacterial Community Composition in Brazilian Anthrosols and Adjacent Soils Characterized Using Culturing and Molecular Identification. Microbial Ecology 58, 23-35.
Paz-Ferreiro, J., Gasco, G., Gutierrez, B., Mendez, A., 2012. Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. Biology and Fertility of Soils 48, 511-517.
Pietikäinen, J., Kiikkila, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89, 231-242.

Pignatello, J.J., Kwon, S., Lu, Y.F., 2006. Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): Attenuation of surface activity by humic and fulvic acids. Environmental Science & Technology 40, 7757-7763.
Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397-420.
Rousk, J., Baath, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. Isme Journal 4, 1340-1351.
Saiya-Cork, K.R., Sinsabaugh, R.L., Zak, D.R., 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. Soil Biology & Biochemistry 34, 1309-1315.
Santos, F., Torn, M.S., Bird, J.A., 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biology & Biochemistry 51, 115-124.
Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A Review of Biochar and Its Use and Function in Soil. Advances in Agronomy, Vol 105 105, 47-82.
Spokas, K.A., Novak, J.M., Venterea, R.T., 2012. Biochar's role as an alternative N-fertilizer: ammonia capture. Plant and Soil 350, 35-42.
Steinbeiss, S., Gleixner, G., Antonietti, M., 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biology & Biochemistry 41, 1301-1310.
Steiner, C., Das, K.C., Garcia, M., Forster, B., Zech, W., 2008. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. Pedobiologia 51, 359-366.
Sudhakar, Y., Dikshit, A.K., 1999. Kinetics of endosulfan sorption on to wood charcoal. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 34, 587-615.
Wardle, D.A., Zackrisson, O., Nilsson, M.C., 1998. The charcoal effect in Boreal forests: mechanisms and ecological consequences. Oecologia 115, 419-426.
Zackrisson, O., Nilsson, M.C., Wardle, D.A., 1996. Key ecological function of charcoal from wildfire in the Boreal forest. Oikos 77, 10-19.

Table 1: Physicochemical properties in soil amended with wood, PyOM and control and two levels of nitrogen (ambient = N0 and increased =N+). Numbers in brackets indicate SD of three field replicates).

Treatments	Wood, N0	Wood, N+	PyOM, N0	PyOM, N+	Control, N0	Control, N+
pH	5.6 (0.8)	5.9 (0.3)	5.2 (0.7)	5.9 (0.3)	5.9 (0.5)	5.8 (0.2)
CEC, mmol kg^{-1} soil	67 (23)	83 (9)	62 (15)	79 (13)	74 (15)	68 (6)
Total C, g kg^{-1} soil	30 (9)	48.1 (3.4)	40.6 (10)	45.4 (5.8)	36.1 (6.6)	36 (3.7)
Total N, g kg^{-1} soil	2.2 (0.6)	3.1 (0.3)	2.4 (0.5)	2.7 (0.4)	2.6 (0.4)	2.6 (0.2)
Average no. of OTUs	4011 (579)	4400 (1011)	4513 (1917)	4354 (990)	3729 (540)	4847 (1051)

Table 2: Spearman's rank correlation between the relative abundances of the eight most abundant bacterial phyla and proteobacterial classes and soil properties in different treatments.

Taxonomic Group	Correlation			
	pH	Total C	Total N	CEC
Acidobacteria	-0.40	-0.21	-0.25	-0.37
Actinobacteria	-0.28	-0.43	-0.29	-0.21
Bacteriodete	0.13	0.60**	0.52*	0.17
Alphaproteobacteria	-0.53*	-0.21	-0.30	0.66**
Betaproteobacteria	0.15	0.41	0.32	0.22
Deltaproteobacteria	0.67**	0.29	0.38	0.52*
Gammaproteobacteria	0.29	0.53*	0.47*	0.33
Planctomycete	-0.33	-0.16	-0.09	-0.17

*Correlation significant at $p<0.05$; ** Correlation significant at $p<0.001$

Figure legends

Figure 1: The enzyme activity of (a) β-1, 4 glucosidase (BG), (b) β-cellobiohydrolase (CBH), (c) β-N-acetylglucosaminidase (NAG) and (d) phenol oxidase (POxy) in the topsoil (0-5 cm) that received input of highly labeled wood or PyOM and no input (control), under ambient N (white) and increased N (grey). For the same enzyme different letters indicate significant differences among treatments ($p<0.05$).

Figure 2: Relative abundance of dominant taxa (phyla *Acidobacteria*, *Actinobacteria*, *Bacteriodete*, *Chloroflex*, *Firmicutes*, *Gemmatimonadetes*, *Plantomycetes*, *Proteobacteria*, *WS3* and others) in the control soils and soil amended either with wood or PyOM under ambient N (N0) and added N (N+) treatment. The error bars correspond to standard error (n=3).

Figure 3: Principal component analysis (PCA) relating microbial communities to organic input – nitrogen treated soils (3 replicates). The percentages in the axis labels represent the percentages of variation explained by the principal coordinates.

Figure 4: Nonmetric multidimensional scaling plots derived from pairwise weighted Unifrac distances between soils under different inputs. The circles, squares and triangles denote soils amended with PyOM, wood and control, respectively. The filled symbols represent soil under added N treatment and empty symbols denote soils under ambient N treatment.

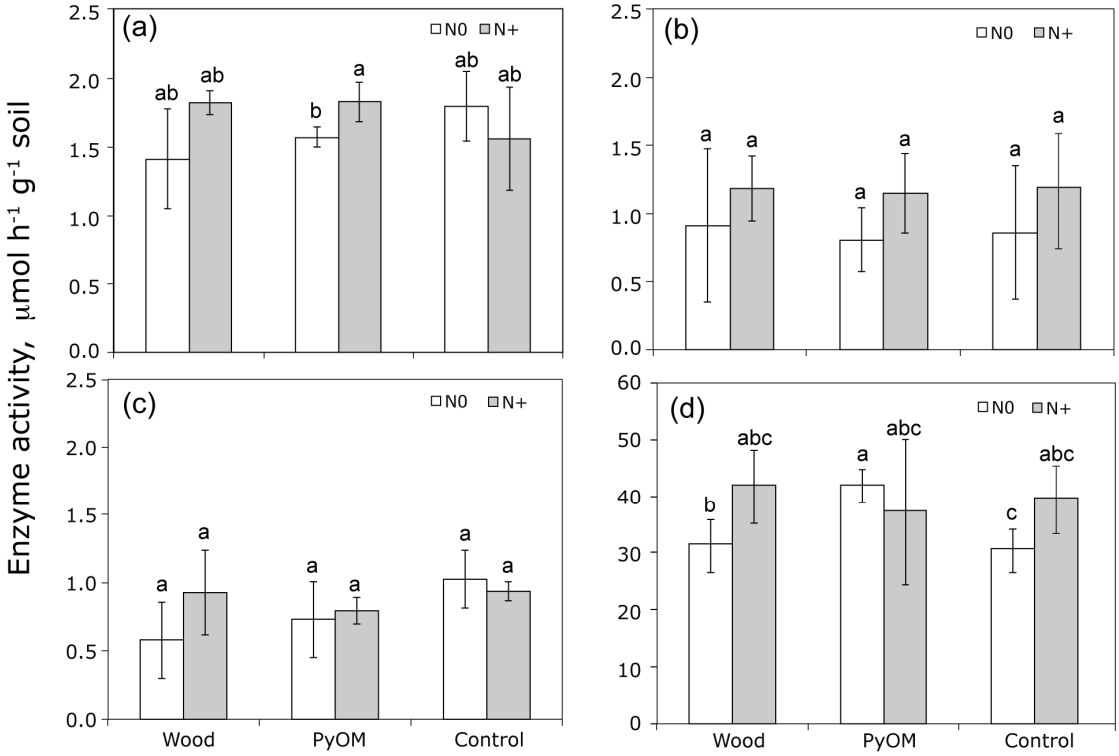

Figure 1

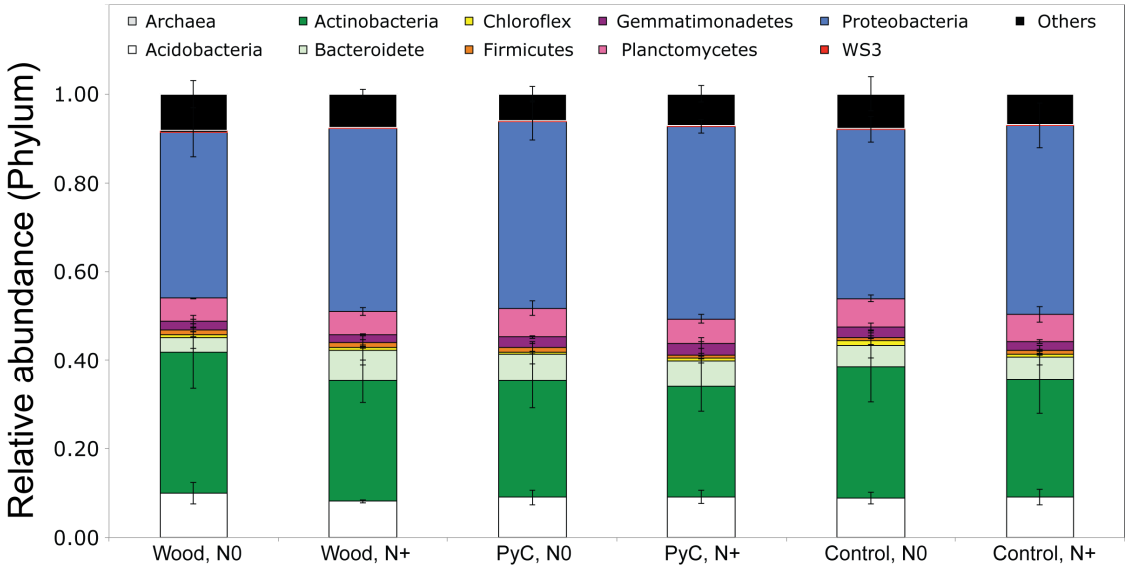
Archaea
Actinobacteria
Chloroflex
Gemmatimonadetes
Proteobacteria
Others
Acidobacteria
Bacteroidete
Firmicutes
Planctomycetes
WS3
Relative abundance (Phylum)
1.00
0.80
0.60
0.40
0.20
0.00
Wood, N0
Wood, N+
PyC, N0
PyC, N+
Control, N0
Control, N+

Figure 2

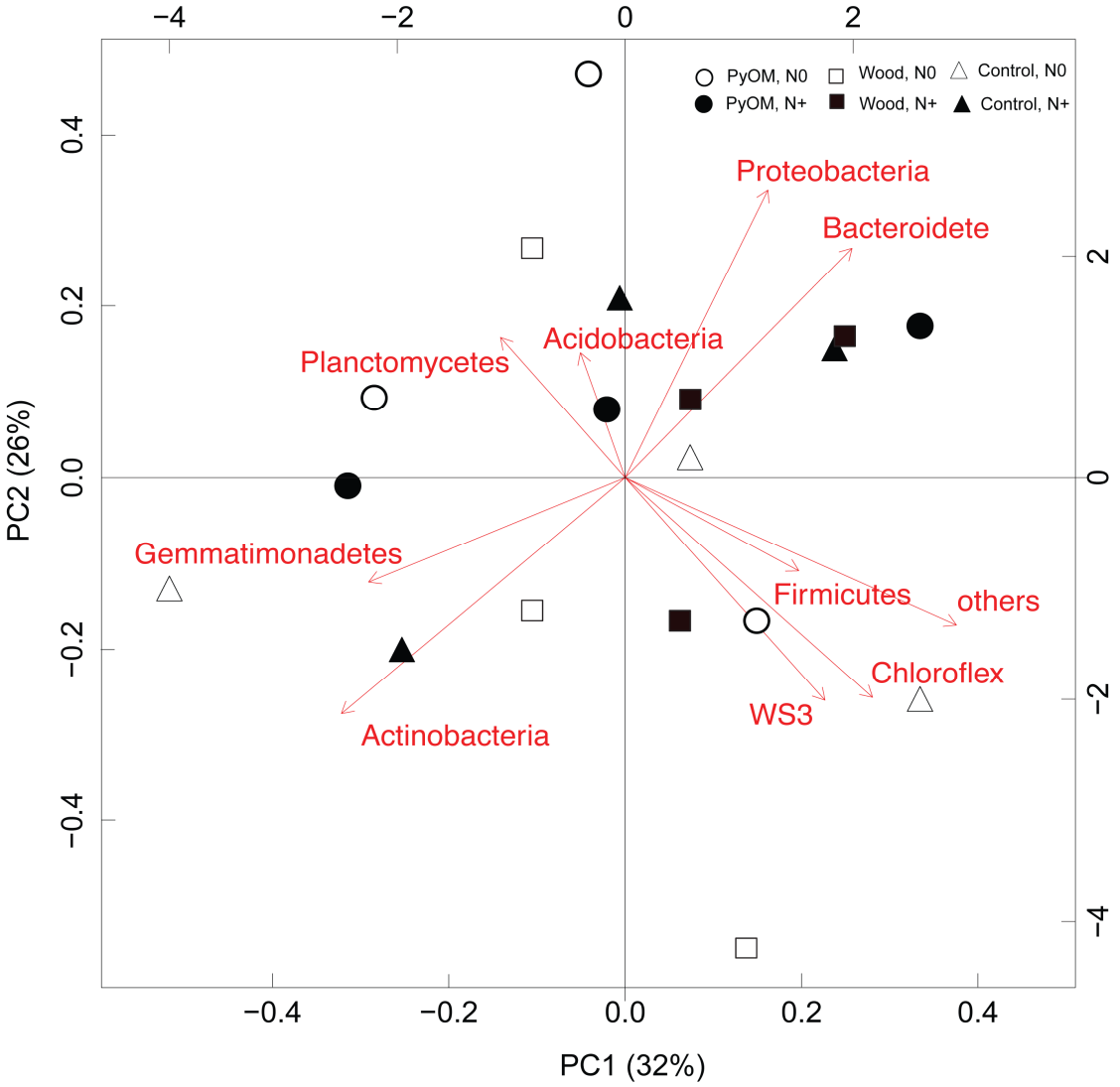
PyOM, N0
PyOM, N+
Wood, N0
Wood, N+
Control, N0
Control, N+
Proteobacteria
Bacteroidete
Acidobacteria
Planctomycetes
Gemmatimonadetes
Firmicutes
others
Chloroflex
WS3
Actinobacteria
PC1 (32%)
PC2 (26%)
−4
−2
0
2
0.4
0.2
0.0
−0.2
−0.4
−0.4
−0.2
0.0
0.2
0.4

Figure 3

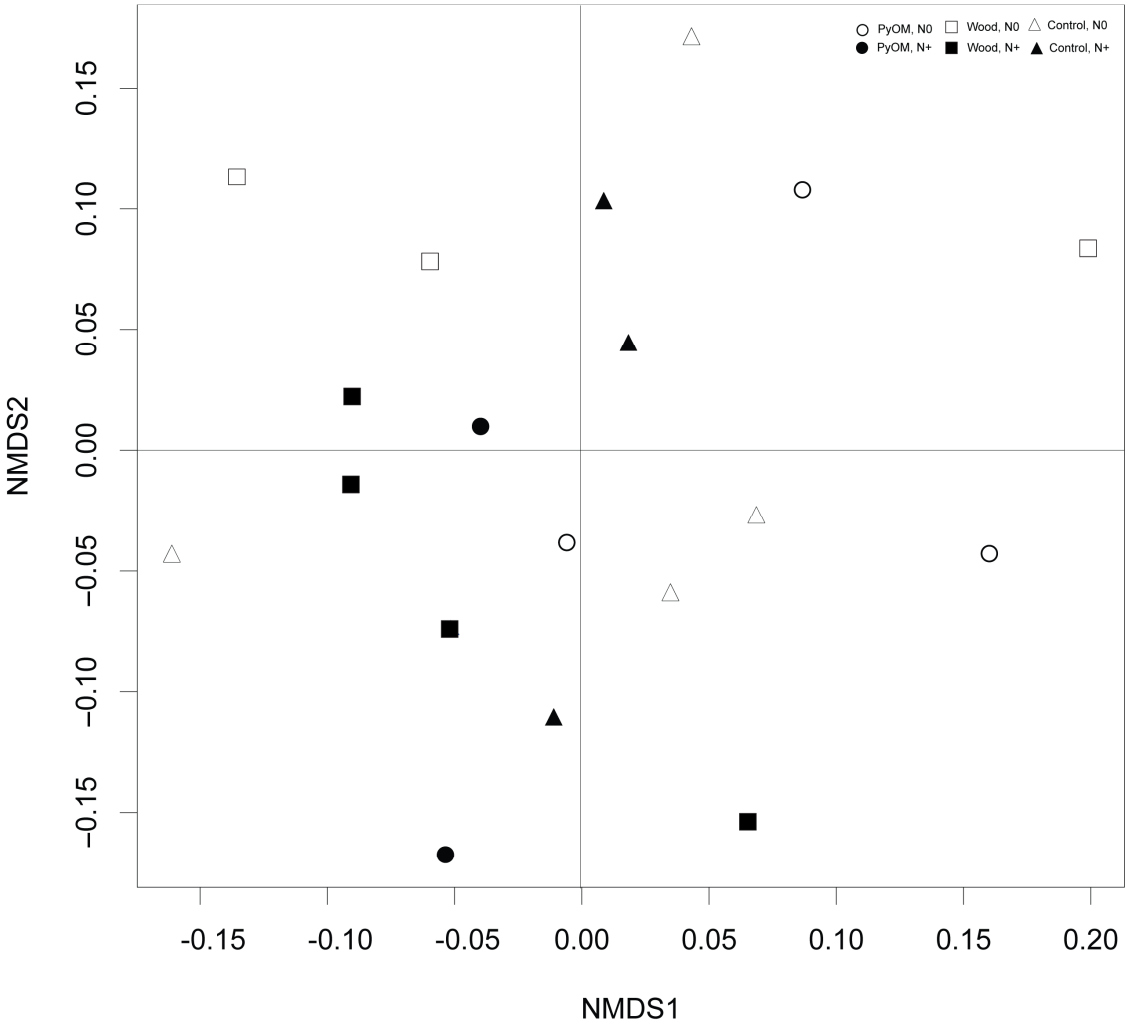
PyOM, N0
Wood, N0
Control, N0
PyOM, N+
Wood, N+
Control, N+
NMDS2
0.15
0.10
0.05
0.00
−0.05
−0.10
−0.15
-0.15
-0.10
-0.05
0.00
0.05
0.10
0.15
0.20
NMDS1

Figure 4

Manuscript IV

Stable isotopic analysis of pyrogenic organic matter in soils by liquid chromatography-isotope-ratio mass spectrometry of benzene polycarboxylic acids

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Stable isotopic analysis of pyrogenic organic matter in soils by liquid chromatography–isotope-ratio mass spectrometry of benzene polycarboxylic acids

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Pyrogenic organic matter (PyOM), the incomplete combustion product of organic materials, is considered stable in soils and represents a potentially important terrestrial sink for atmospheric carbon dioxide. One well-established method of measuring PyOM in the environment is as benzene polycarboxylic acids (BPCAs), a compound-specific method, which allows both qualitative and quantitative estimation of PyOM. Until now, stable isotope measurement of PyOM carbon involved measurement of the trimethylsilyl (TMS) or methyl (Me) polycarboxylic acid derivatives by gas chromatography–combustion–isotope ratio mass spectrometry (GC-C-IRMS). However, BPCA derivatives can contain as much as 150% derivative carbon, necessitating post-analysis correction for the accurate measurement of δ^{13} C values, leading to increased measurement error. Here, we describe a method for δ^{13} C isotope ratio measurement and quantification of BPCAs from soil-derived PyOM, based on ion-exchange chromatography (IEC-IRMS). The reproducibility of the δ^{13} C measurement of individual BPCAs by IEC-IRMS was better than 0.35‰ (1σ). The δ^{13} C-BPCA analysis of PyOM in soils, including at natural and artificially enriched ^{13} C-abundance, produced accurate and precise δ^{13} C measurements. Analysis of samples that differed in δ^{13} C by as much as 900‰ revealed carryover of <1‰ between samples. The weighted sum of individual δ^{13} C-BPCA measurements was correlated with previous isotopic measurements of whole PyOM, providing complementary information for bulk isotopic measurements. We discuss potential applications of δ^{13} C-BPCA measurements, including the study of turnover rates of PyOM in soils and the partitioning of PyOM sources based on photosynthetic pathways. Copyright © 2011 John Wiley & Sons, Ltd.

Fire is a major controller of carbon (C) cycling in terrestrial ecosystems, by converting plant biomass into atmospheric CO_2 and by contributing incompletely combusted biomass or pyrogenic organic matter (PyOM) to soils.[1,2] PyOM is ubiquitous in the environment and it can be a sizable fraction of the stable portion of soil C.[3–5] Recent interest in a better understanding of the chemical properties, stocks, and turnover rates of PyOM has been driven by the potential role of PyOM as a stable C sink for atmospheric CO_2,[6,7] as a sorbent for pollutants,[8,9] and to improve plant fertility in highly weathered soils.[10,11] Furthermore, the amount of PyOM added to the environment is expected to increase because of predicted increases in wildfire frequency and intensity with a warmer future climate[12,13] and significant contributions of biochar produced by the energy industry as a byproduct of low-temperature biomass pyrolysis.[14,15]

One of the challenges to better understanding the roles of PyOM in the environment is its methodological assessment, especially in complex, C-rich matrices like soil. PyOM represents a continuum of materials, whose structures are affected by the heat treatment temperature and conditions of combustion.[16–19] Recent approaches to the characterization of PyOM in environmental samples are diverse, and include spectroscopy,[20–22] analytical pyrolysis,[23,24] and molecular markers such as levoglucosan[25] and the benzene polycarboxylic acids.[26,27] A large, multi-laboratory study by Hammes *et al.*[28] compared seven methods of PyOM analysis on various environmental samples and observed that PyOM produced by wildfire conditions were well estimated by the benzene polycarboxylic acid (BPCA) molecular biomarker method. The BPCA approach was the only method that could reliably quantify PyOM and simultaneously characterize the chemical structures of PyOM materials in soil matrices. Hammes *et al.*[28] cautioned that the BPCA approach might underestimate the highly condensed soot part of the PyOM continuum and also include BPCA contributions from coal and shale. Recent advances in the BPCA extraction method,[29] especially the addition of systematic standardization procedures,[30] have addressed the inter-laboratory variability observed for BPCA.[28]

During the last decade, the BPCA approach has been developed into a robust method to quantify the amount of PyOM materials in soils.[31–35] BPCAs are produced through the oxidation of substituted aromatic organic C and are, thus,

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a measure of the main building blocks of most PyOM materials. Consequently, BPCAs can provide qualitative information on the degree of aromatic condensation of the PyOM present.[30,35] As the turnover rates of PyOM in the environment have been related to the heat treatment temperature and resulting degree of aromatic condensation,[16,35] the BPCA approach can provide a clearer picture of PyOM degradability and mobility in the environment. When the BPCA approach is combined with stable and radiogenic C isotope tracers (i.e. ^{13}C and ^{14}C), the movement, loss and transformation of PyOM can be directly measured *in situ*.[36]

Initial $\delta^{13}C$ measurements of PyOM molecular markers have relied on gas chromatography–combustion–isotope-ratio mass spectrometry (GC-C-IRMS) of trimethylsilyl and methyl derivatives of BPCAs.[37] Unfortunately, GC-C-IRMS exhibits a number of shortcomings when applied to the measurement of stable isotopic composition of non-volatile BPCAs, primarily due to the need for derivatization. For example, the TMS and methyl derivatives of mellitic acid (benzene hexacarboxylic acid; B6CA) add 18 or 6 C atoms, respectively, to the twelve C atoms present in B6CA. Because all of the C present in the derivatized molecule is ultimately oxidized to CO_2, and therefore contributes to the final $\delta^{13}C$-CO_2 measurement, significant correction factors must be applied to generate reasonable estimates of $\delta^{13}C$-BPCA. Moreover, BPCA derivative interactions,[30] isotope dilution, kinetic isotope effects, and incomplete derivatization associated with GC-C-IRMS, result in increased $\delta^{13}C$ measurement error.[38]

High-performance liquid chromatography–isotope-ratio mass spectrometry (HPLC-IRMS) may be a suitable alternative for the measurement of $\delta^{13}C$-BPCA. LC-IRMS would not require the derivatization of BPCAs prior to analysis, potentially providing better overall accuracy and precision without the requirement for correction factors. While two suitable methods for BPCA analysis by HPLC have been demonstrated,[29,35] neither method is suitable for transfer to the current generation of LC-IRMS instrumentation, as separation methodologies for LC-IRMS cannot utilize organic solvents in the mobile phase.[39] In contrast, anion-exchange chromatography is particularly well suited to the separation of water-soluble ionic compounds without the use of organic solvents. Several applications of LC-IRMS have adopted ion-exchange chromatography (IEC-IRMS) for the separation of ionic organic compounds including carbohydrates,[40] amino acids,[41] and amino sugars.[42] IEC-IRMS is suitable for application to a wide range of other organic acids,[43,44] including aromatic carboxylic acids.[45]

In this study, we present an IEC-IRMS method for the measurement of $\delta^{13}C$-BPCA following hydroxide gradient separation of individual BPCAs by anion-exchange chromatography, taking advantage of the ionic properties of benzene polycarboxylic acids. The accuracy and precision of this method were evaluated using a series of plant-derived PyOM and soil samples, representing a range of sample matrices and isotopic composition. The samples included (1) PyOM from both C_3- and C_4-plants, (2) PyOM with and without a ^{13}C-tracer (natural abundance and artificially enriched), (3) Mollisol soil from a C_3-dominated ecosystem, (4) artificial mixtures of C_4-PyOM and C_3-Mollisol soils, and (5) Spodosol soil from a C_3-dominated ecosystem. We briefly discuss the suitability of IEC-IRMS to potential applications of $\delta^{13}C$-BPCA measurements.

EXPERIMENTAL

Chemicals

Eight benzene polycarboxylic acids are used as biomarkers of PyOM (Fig. 1). Pure benzene polycarboxylic acids, including benezenetricarboxylic acid (B3CA), 1,2,3-B3CA (hemimellitic acid), 1,2,4-B3CA (trimellitic acid), 1,3,5-B3CA (trimesic acid); benzenetetracarboxlic acid (B4CA),1,2,4,5-B4CA (pyromellitic acid); and benzenehexacarboxylic acid (B6CA; mellitic acid), were purchased from Sigma-Aldrich (St. Louis, MO, USA). Benzenepentacarboxylic acid (B5CA) was supplied by Alfa Aesar (Heysham, UK). Two of the benzenetetracarboxylic acids (B4CA), 1,2,3,5-B4CA (mellanophanic acid) and 1,2,3,4-B4CA

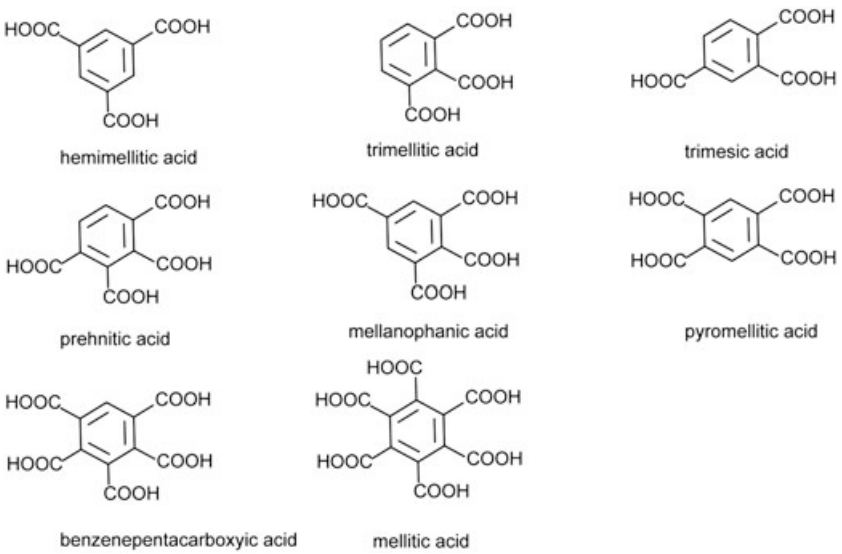

Figure 1. Structure and nomenclature of benzene polycarboxylic acid biomarkers of PyOM.

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(prehnitic acid), were not commercially available. A sodium hydroxide stock solution (50% w/w) was supplied by Acros Organics (Geel, Belgium). HPLC-grade orthophophoric acid and sodium persulfate originated from ThermoFisher Scientific (Fair Lawn, NJ, USA).

Sample descriptions

An overview of the samples used in this study is given in Table 1. The pyrolysis treatment for all PyOM materials examined in this study (i.e., chestnut, pine and maize) was performed at a constant temperature of 450 °C for 6 h under N_2.[21] The standard PyOM material analyzed here was produced by pyrolyzing chestnut (*Castanea sativa*) wood. The $^{13}C/^{15}$ N-labeled pine PyOM was produced by pyrolysing 2–3-year-old Ponderosa pine (*Pinus ponderosa*) saplings which were grown in a stable isotope labeling chamber at the University of California, Davis in 2001.[46] The maize (*Zea mays*) PyOM was sampled at maturity in an experimental field in Rotthalmünster, Germany.[47] The maize stem was separated from the leaves and roots and chopped into 1–3 cm particles prior to charring.

Northern temperate forest soil samples (A horizon, 0–5 cm depth) were collected in 2009 from an on-going fire manipulation experiment located at the University of Michigan Biological Station (UMBS), Pellston, MI, USA (45°35′N, 84°43′W). The 1 ha forested plot sampled at UMBS was last burned in 1911, and is located on an outwash plain derived from glacial drift. The UMBS soils are well-drained, sandy, mixed frigid Entic Haplothods (Rubicon series), consisting of 92 g kg^{-1} sand and 1 g kg^{-1} clay.[48] Two composite soil samples (A and B) were included in this analysis, each from the same forest plot. Soil samples were collected at three sampling points equally spaced along a 120 m transect in the plot. Soil samples were formed by combining five soil cores taken at a 1 m radius around each sampling point. In this study, Spodosol soil A is a composite sample from one sampling point in a transect, and contains 15.89 ± 0.06 g kg^{-1} C. Spodosol soil B was formed by combining soil composites from all sampling points in one single transect, and contains 11.92 ± 0.15 g kg^{-1} C. The soils were air-dried and sieved (<2 mm) before analysis.

Mollisol soils (Chernozem) were sampled at 20–60 cm depth in the Hildesheim-Braunschweig region, Harsum, Germany[49] and used as a reference material in pyrogenic organic matter studies.[28,30,34] These soils are characterized as light sandy clay with 19 wt% clay content and 20.1 g C/kg.

In order to assess the ability of the method to detect ^{13}C-BPCA differences for natural samples, we mixed C_4-maize-PyOM to C_3-Mollisol at 1:1 C%. Ground samples were gently mixed, rewetted to field capacity and incubated for 1 day. The mixed samples were dried and milled again prior to analysis. The measured values were compared with the expected values, calculated as the weighted average of ^{13}C-BPCA from maize-PyOM and Mollisol soil.

BPCA extraction

We used the revised BPCA method as described by Schneider *et al.*,[30] which was first described by Glaser *et al.*[26] and later modified by Brodowski *et al.*[27] Briefly, the revised method replaces hydrochloric acid in the digestion procedure with trifluoroacetic acid (TFA) prior to the oxidation of samples with HNO_3 and phthalic acid as the internal standard. Soil and PyOM samples were homogenized prior to extraction and completed in triplicate following Brodowski *et al.*[27]

Chromatography

Individual BPCAs were separated at 30 °C on a Dionex IonPac® AS11 column (2 × 250 mm) with an AG11 guard column (Dionex Corp., Sunnyvale, CA, USA) using a sodium hydroxide gradient (NaOH) delivered by a Surveyor HPLC pump (Thermo Scientific, Madison, WI, USA). The solvents were (A) deionized water and (B) 100 mM NaOH. The linear gradient profile was (B in A): 0–1 min, 30%; 1–10 min, 30–35%; 10–20 min, 35–40%; 20–45 min, 40–80%; 45–60 min, 80–100%; 60–70 min, 100%. The flow rate was 350 µL min^{-1}. Sample injection was automated and performed with a 5 or 10 µL injection loop. The targeted sample amount was 150–350 ng C for B5CA and B6CA.

All solvents were degassed by sonication under reduced pressure for 1 h. In the case of the NaOH solvent, the carbonate-free NaOH stock solution (50% w/w) was not added until the water had first been degassed (1 h); the prepared solution was then degassed for an additional 15 min. During use, solvents were bubbled with a continuous stream of helium (99.9999% purity, Praxair, Inc., Sacramento, CA, USA) to prevent regassing. This procedure helped to reduce CO_2 contamination of the NaOH solvent and maintain a relatively low background of CO_2 in the IRMS system.

Table 1. PyOM and soils used for the evaluation of δ^{13}C-BPCA analysis by IEC-IRMS

Sample	Description	Organic C (g kg^{-1})
PyOM[a]		
Castanea sativa PyOM[b]	Chestnut wood, Ticino, Switzerland	774
Zea mays (C_4) PyOM	Corn stem, Rotthalmünster, Germany	755
Pinus ponderosa PyOM	Pine wood, California, USA	779
Soils		
Mollisol[c]	Hildesheim, Germany, 20–60 cm depth	19.3
Spodosol	Pellston, MI, USA, 0–5 cm depth	15.9 ('A'), 11.9 ('B')

[a]All PyOM materials were charred at 450 °C under N_2 environment (Hammes *et al.*[21]).
[b]Hammes *et al.*[21,28]
[c]Schmidt *et al.*[48] Hammes *et al.*[28]

The system was re-equilibrated for 15 min at initial conditions prior to each analysis. After every ten samples, the column was flushed with 200 mM NaOH for 1 h and allowed to equilibrate for 30 min prior to subsequent analysis. After extensive use for the analysis of soil samples, the quality of the separation became reduced and the retention time shortened, probably due to the accumulation of metals or hydrophilic anions on the column. The column was then sequentially washed with (1) 2 M HCl, (2) water, and (3) 2 M NaOH, for 1 h each, and re-equilibrated prior to subsequent use. Column cleanup was performed if average retention times had shifted more than 10 s, and expected retention was not re-established by the hydroxide wash.

For six of the eight BPCAs, compounds were identified based upon retention times through the use of a prepared mixture of pure compounds. For the two commercially unavailable B4CAs (1,2,3,4-B4CA and 1,2,3,5-B4CA), retention times and elution order were predicted from their published pK_a values,[50] and comparison with GC data from soil samples and standards with variable individual B4CA content.

Combustion-oxidation and isotope-ratio mass spectrometry

After separation, the column eluent enters an oxidation interface, LC Isolink (Thermo Scientific, Bremen, Germany), where it is mixed with sodium peroxidisulfate (0.8 M) and phosphoric acid (1.5 M), separately pumped at 60 and 90 μL min^{-1} respectively. Individual BPCA peaks are then quantitatively oxidized to CO_2 as the mobile phase flows through an oxidation reactor heated to 99.9 °C. Each CO_2 peak is transferred from aqueous phase to He carrier gas (2 mL min^{-1}) through a separation membrane. Following water removal using a two-stage Nafion™ dryer, the individual CO_2 peaks are transferred to an isotope-ratio mass spectrometer (Finnigan Delta^{Plus} Advantage, Thermo Electron, Bremen, Germany) through an open split.

During analysis, three pulses of CO_2 (99.995% purity, Praxair Inc., Sacramento, CA, USA) of known $\delta^{13}C$ value, each 20 s in duration, were introduced into the IRMS instrument and used to calculate the provisional $\delta^{13}C$ values of the sample peaks (expressed in ‰, referenced vs. VPDB). Post-analysis, the measurements were standardized with a prepared mixture of five BPCAs (BPCA-MIX) of varying concentration (50–500 ng C/μL each). The BPCA-MIX was analyzed every five samples to allow for correction for instrument drift and used to evaluate linearity. Peak area integration parameters were set to a slope (mV/s) of 0.1 with a dynamic background algorithm with a step-width of 75 points[51] to account for any changes in the background (ISODAT version 2.5; Thermo Scientific, Bremen, Germany).

For each BPCA, a final shift to $\delta^{13}C$ values obtained through elemental analyzer-IRMS (EA-IRMS) was used to compensate for any potential offset produced by stationary phase interactions during the IEC-IRMS measurement[52] (see Table 2). Briefly, 350 μg C of each pure compound was weighed into tin capsules, combusted and oxidized to CO_2 in an elemental analyzer, and purified and analyzed using an isotope-ratio mass spectrometer (20–20, Sercon, Crewe, UK). During analysis, the samples were interspersed with several replicates of two different laboratory standards (nylon, $\delta^{13}C$ value = −27.41, and glutamic acid, $\delta^{13}C$ value = +43.74) that had been previously calibrated against NIST Standard Reference Materials (USGS-40 and USGS-41, National Institute of Standards and Technology, Gaithersburg, MD, USA). The long-term standard deviation is ≤0.2‰ for $\delta^{13}C$ values. $\delta^{13}C$ values obtained by EA-IRMS were assigned to each component of the BPCA-MIX.

RESULTS AND DISCUSSION

Chromatography

Sufficient separation of all B3CA through B6CA, except 1,2,4-B3CA, was possible across a 30–100 mM sodium hydroxide gradient (mean resolution, R >1.5; Fig. 2). The co-elution of the B3CAs with other di- and tricarboxylic organic acids presents the greatest obstacle to complete BPCA separation by IEC. Despite testing a range of chromatographic conditions, we were unable to completely separate 1,2,4-B3CA from a co-eluting non-BPCA compound (mean resolution, R = 1.1). We found the non-BPCA compound to be more abundant in the PyOM standards than in the soils, where the compound produces a small shoulder on the 1,2,4-B3CA peak (Fig. 2). The presence of this shoulder will increase the measurement error associated with 1,2,4-B3CA, as the $\delta^{13}C$ measurement of overlapping regions will represent a mixture of the two compounds. The magnitude of this effect will depend upon on the relative concentration of the two compounds. Separation of the remaining BPCA did not differ significantly between the extracts of PyOM and soils (Fig. 2). The concentrations of non-BPCA organic acid concentrations were greater in BPCA extracts of woody PyOM (*C. sativa*, *P. ponderosa*) than in those of non-wood-PyOM (*Zea mays*) and soils (Fig. 2(B)).

Further developments in sample purification, especially the removal of non-BPCA carbon, will be critical to simplifying the separation of all B3CAs. We noted that the quality of separation was greatly affected by the presence of other organic anions and changes in column condition; this has also

Table 2. Comparison of $\delta^{13}C$ values (‰,'per mil') obtained by EA-IRMS and IEC-IRMS analysis of individual BPCAs (σ, n = 3). B5CA was not measured (n.m.) by EA-IRMS due to limited availability. 1,2,3-BPCA and 1,2,4-BPCA were measured under full and partial separation by IEC-IRMS

	1,2,3-BPCA	1,2,4-BPCA	1,3,5-BPCA	1,2,4,5-BPCA	B5CA	B6CA
EA-IRMS	−21.75 ± 0.03	−27.91 ± 0.02	−25.90 ± 0.11	−26.42 ± 0.02	n.m.	−26.99 ± 0.02
IEC-IRMS	−23.01 ± 0.16	−29.63 ± 0.44	−27.04 ± 0.60	−25.97 ± 0.70	−29.67 ± 0.74	−26.28 ± 0.22

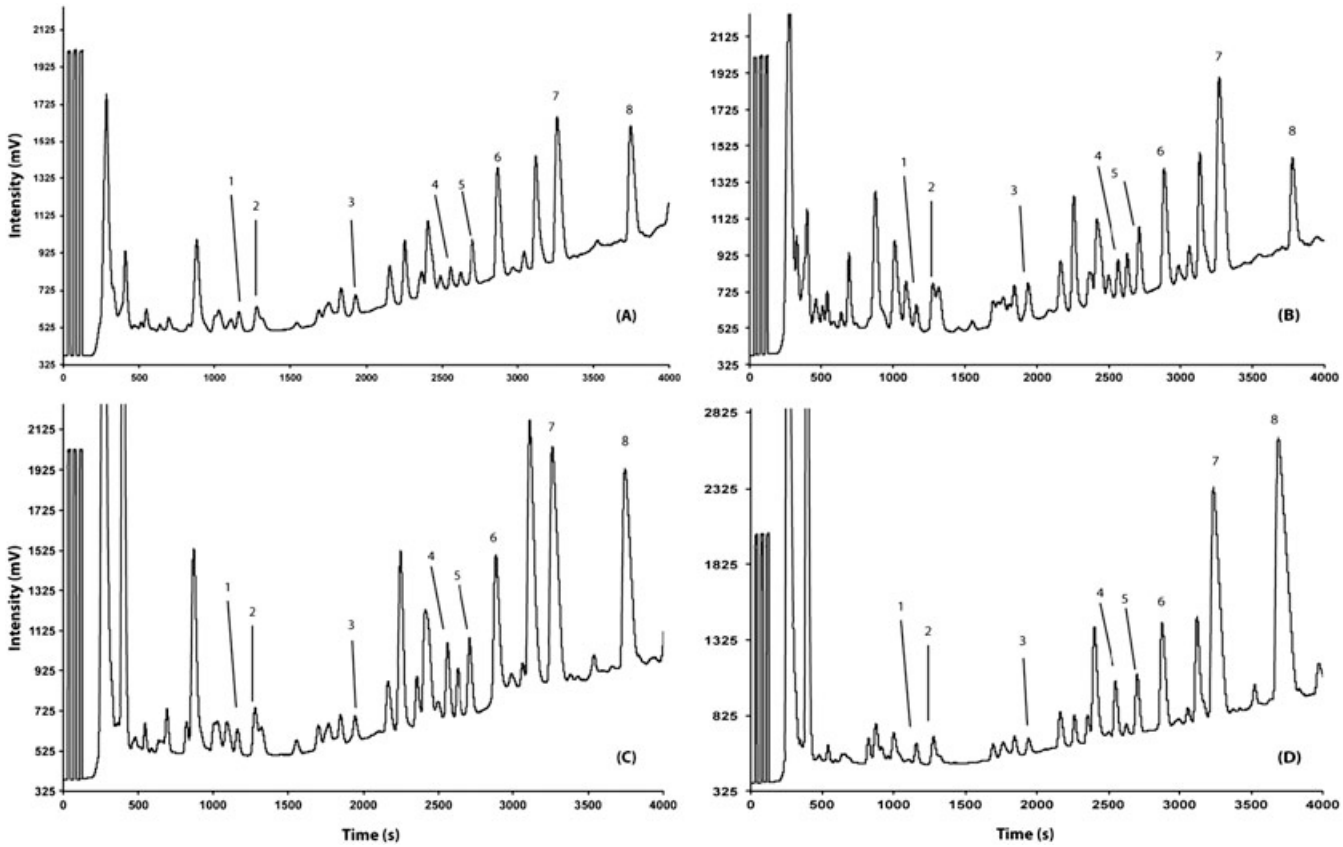

Figure 2. Chromatographs of BPCAs from (A) *Zea mays* PyOM, (B) *Pinus ponderosa* PyOM, (C) Spodosol soil (Soil A), and (D) Mollisol soil. 1: 1,2,3-B3CA, 2: 1,2,4-B3CA, 3: 1,3,5-B3CA, 4: 1,2,4,5-B4CA, 5: 1,2,3,5-B4CA, 6: 1,2,3,4 B4CA, 7: B5CA, 8: B6CA.

been shown to be problematic for more traditional HPLC-BPCA methods.[29] In addition, care must be exercised in the removal of polyvalent cations from soil samples during sample preparation.(e.g.[42]). High concentrations of these ions in the sample matrix may also greatly affect column retention through local electrostatic effects.

Some researchers have previously expressed concern over the use of hydroxide-based separations in IEC-IRMS, due to the loss of precision with potentially high CO_2 backgrounds. However, the careful preparation of solvents (as described above) and use of a relatively high acid flow rate (90 μL min^{-1}) resulted in a low background across a strong OH^- gradient (*m/z* 44 background: 400–900 mV; recommended maximum background[53]: <1 V). Furthermore, significant changes in the precision of δ^{13} C measurements with increasing hydroxide concentrations were not observed (Table 2).

Quantification

The relative abundances of Mollisol soil BPCAs measured by IEC-IRMS compared favorably with those from GC-FID measurements (Fig. 3). The measured proportions of B5CA and B6CA from this soil by the two methods differed by less than 1%, while the relative abundance of B3CA (excluding 1,3,5-B3CA) was found to be marginally higher (~ +2.8%) and B4CA slightly lower (~ −1.7%) when measured by IEC-IRMS than by GC-FID. The concentrations of the individual BPCAs measured by IEC-IRMS were ≥1:1 of those measured by GC-FID (Fig. 4). Notably, the concentrations of BPCAs of Spodosol soils were higher when quantified by IEC-IRMS. This may be due to sample-specific differences in the completeness of BPCA derivatization in preparation for GC-FID analysis. However, the observed differences in quantification between soil types suggest the use of a native concentration standard until the underlying reasons for the differences are determined. 1,3,5-B3CA was not identified in the GC-FID analysis of the soils, yet was quantifiable by IEC-IRMS (range: 0.58–1.29 μg C mL^{-1}).

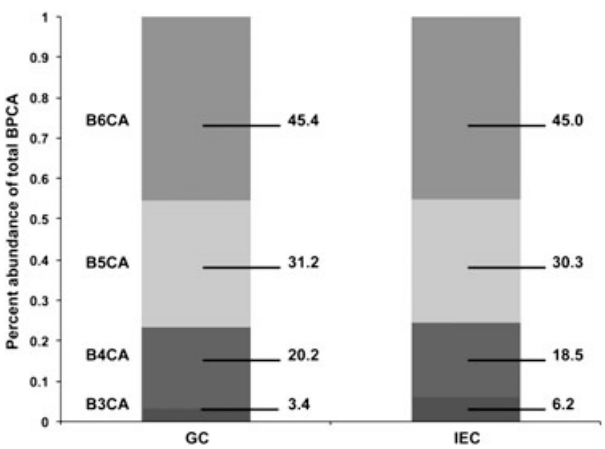

Figure 3. Relative abundance of each class of BPCAs of Mollisol soil (n = 2, with two replicates) as measured by IEC-IRMS and GC-FID.

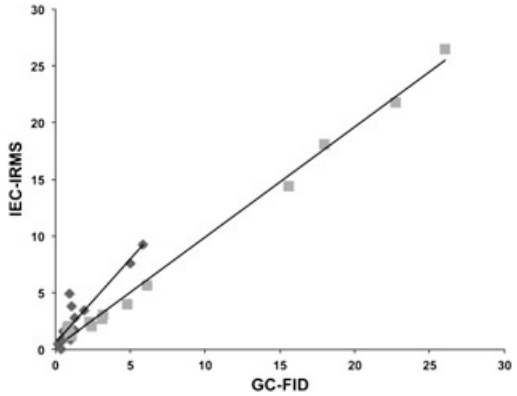

Figure 4. Quantification of BPCAs (μg C/g soil) by IEC-IRMS relative to GC-FID of Mollisol (n = 2, with two replicates, squares; 0.9726x + 0.429) and Spodosol soils (A & B; n = 2, with two replicates, diamonds; 1.4763x + 1.3504). Each point represents the mean value of individual BPCAs.

We did not observe a strong linear effect of injection amount on the δ^{13} C measurements. A series of 15 measurements of 1,2,3-B3CA, ranging from 75 to 525 ng C per injection, produced a standard deviation of <0.26‰ (1 SD, δ^{13}C range: –22.17, –23.15; R^2 = 0.02) with no relationship between δ^{13}C value and 1,2,3-B3CA concentration (linear slope = −0.0002). Flow-injection analysis (direct injection) of the other BCPA-MIX constituents also did not reveal a significant linear relationship from 100 to 500 ng C per injection (α = 0.05). However, the use of a concentration-variable reference mixture would be wise in order to account for potential changes in the combustion interface, as well as providing an efficient basis for validating the quantification of BPCA concentration.

Accuracy and precision

Overall, precision estimates of the δ^{13}C-BPCA values by IEC-IRMS compare favorably with those by GC-C-IRMS. The precision (1σ, n = 3) of δ^{13}C-BPCA values from pure compounds (BPCA-MIX) by IEC-IRMS was ≤0.74‰ (Table 2), while that of sample duplicates was ≤0.2‰ (Table 3). The precision estimates (mean standard error) reported by Glaser and Knorr[37] were ≤2.4‰ and ≤1‰ for TMS and Me derivatives, respectively. The precision of δ^{13}C-BPCA values from duplicate samples (1σ, n = 2) of natural-abundance PyOM and Mollisol soils was ≤0.36‰, and ≤8.8‰ for the ^{13}C-labeled *P. ponderosa* PyOM.

Raw δ^{13}C measurements of B4CA-B6CA obtained by IEC-IRMS matched well those obtained by EA-IRMS, while IEC-IRMS measurements of B3CA were generally ~1‰ less than those by EA-IRMS (Table 1). In addition, IEC-IRMS of BPCAs from C_4-PyOM (*Zea mays*), C_3-Mollisol soils, and artificial mixtures produced an expected range of δ^{13}C values (Table 3). Notably, the weighted sum of the IEC-IRMS measurement of individual BPCAs approximated the bulk EA-IRMS measurements of the standard reference PyOM materials (within 5‰), both at natural abundance and when artificially enriched in ^{13}C (Table 3). We did not expect these measurements to exactly match the measurement of the bulk PyOM or soils, as these materials also contain non-BPCA carbon; however, this outcome was encouraging. The accuracy of the δ^{13}C-BPCA values measured by GC-C-IRMS reflects the contribution of non-analyte carbon to the δ^{13}C measurement (as high as 11‰[37]); derivatization procedures for δ^{13}C-BPCA measurements result in products containing up to 1.5 times as much non-analyte carbon and these require appropriate correction procedures (TMS and Me derivatives[37]). The measurement error of δ^{13}C values by GC-C-IRMS is propagated as the final estimate of the δ^{13}C value relies on measurements of both the derivatization agent and the analyte. Further, kinetic isotope effects associated with derivatization of BPCAs may also contribute significant error, as reported for other GC-C-IRMS applications.[38,52]

Limiting carryover, or the incomplete removal of analytes between samples, is essential for stable isotope analysis, especially when measurements include artificially enriched samples. Carryover between samples of differing δ^{13}C values was not significant for natural abundance and ^{13}C-enriched PyOM. The difference between individual δ^{13}C-BPCA values from *C. sativa* PyOM measured after ^{13}C-labeled *P. ponderosa* PyOM ranged from −0.73‰ (1,3,5-B3CA) to 0.40‰ (1,2,4,5-B4CA), which is well within the measurement error for the ^{13}C-enriched PyOM. This feature will be critical for partitioning the relative contribution of multifarious PyOM sources to sample δ^{13}C-BPCA.

Applications

While estimates of PyOM contents and transformations using BPCA provide an important measure of PyOM dynamics, researchers have shown that some BPCAs may derive from non-PyOM sources.[37,54] Consequently, the improved efficacy of combining BPCAs with stable isotope tracers (e.g. ^{13}C-PyOM) allows researchers to directly follow PyOM-derived

Table 3. δ^{13}C measurement of duplicate preparations of PyOM and mineral soils (‰,'per mil'). Sum total represents the weighted sum of the individual BPCA measurements. Reproducibility (σ) of sample duplicates was ±0.36‰ for natural abundance samples, and ±8.8‰ for the *P. ponderosa* PyOM

BPCA	B3CA	B4CA	B5CA	B6CA	Sum	EA-IRMS
Castanea sativa PyOM	−29.72	−28.57	−28.71	−28.24	−29.05	−27.4
Pinus ponderosa PyOM	+804.25	+843.03	+888.17	+821.9	+839.91	+844.79
Zea mays (C_4) PyOM	−15.79	−14.03	−13.84	−14.83	−14.27	n.m.
C_3 Mollisol	−27.42	−26.12	−25.31	−25.70	−25.27	n.m.
C_3 Mollisol + *Zea mays* PyOM (expected)	−24.41	−20.73	−20.02	−21.72	−21.72	n.m.
C_3 Mollisol + *Zea mays* (C_4) PyOM (measured)	−23.08	−20.03	−19.27	−19.31	−20.42	n.m.

n.m.: not measured

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BPCAs in soils, sediments, and leachates without concern for non-PyOM-derived BPCAs. The IEC-IRMS approach significantly improves both the reliability and the accuracy of isotopic BPCA determinations by eliminating the complications related to derivatization using the conventional GC-C-IRMS approach.

The use of BPCA in association with highly enriched ^{13}C-labeled PyOM provides for a robust quantitative and qualitative measure of the decomposition and transport dynamics of PyOM in terrestrial and marine ecosystems. Due to the relatively long, century-scale turnover rates of PyOM, the utility of tracer-level studies would be most evident with short to decade-long studies. However, differences in the natural ^{13}C abundance signature of PyOM (i.e. C_3/C_4 photosynthetic pathways) may provide an opportunity to examine longer time periods of PyOM dynamics.

Researchers have utilized ^{13}C natural abundance signatures for several specific compounds, including lignin,[55,56] polysaccharides,[57] or *n*-alkanes[58] to evaluate their decay rate and their related stabilization mechanisms in soil. Specifically for lignin, several researchers have observed patterns in lignin monomers, and associated these relative changes to corresponding decomposition patterns.[59] Like changes in lignin monomers, the BPCA approach can provide a portrait of PyOM composition by measuring changes in BPCAs. The ^{13}C-BPCA approach will facilitate the investigation of sites with significant wildfire histories, including sequences of vegetation changes between C_4 grassland and C_3 forest. In our study, IEC-IRMS was able to distinguish PyOM of C_3- and C_4-plants, as well as artificial mixtures of C_4-PyOM and C_3-Mollisol soils (Table 3). The measured isotopic values were very close to the expected values (i.e. less than 6% difference to the expected value of the sum), considering the BPCA variability in the PyOM and soils.

The utility of ^{13}C-BPCA using IEC-IRMS should not be restricted to soils or terrestrial sediments, but would be useful for a range of environmental samples. For oceanographic studies, BPCA has been applied to examine the contribution of PyOM to marine dissolved organic matter.[29,60] Furthermore, this approach may also provide utility for the analysis of sediments, since the proportions of BPCAs have been shown to depend on their origin, including terrestrial or oceanic sources.[61,62]

CONCLUSIONS

Suitable methods exist for the analysis of BPCA as PyOM molecular markers, both by GC-FID and by HPLC-DAD. While the isotopic measurement of $\delta^{13}C$-BPCA has been restricted to GC-C-IRMS, IEC-IRMS appears to be a suitable alternative for the measurement of $\delta^{13}C$-BPCA values. The IEC-IRMS determination of $\delta^{13}C$-BPCA values does not require significant post-analysis correction for derivatization carbon, nor does it pose some of the other analytical issues often associated with the GC-C-IRMS of derivatization products. IEC-IRMS is suitable for a range of sample types from PyOM to soils, at both natural and artificial ^{13}C-abundance and should be appropriate for aqueous environmental samples. Potential improvements can primarily be made in sample preparation, specifically improvements in the removal of non-BPCA organic acids.

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REFERENCES

[1] E. D. Goldberg, *PyOM in the Environment: Properties and Distribution*, John Wiley, Chichester, **1985**.
[2] T. A. J. Kuhlbusch. Black carbon and the carbon cycle. *Science* **1998**, *280*, 1903.
[3] M. W. I. Schmidt, A. G. Noack. Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. *Global Biogeochem. Cycles* **2000**, *14*, 777.
[4] J. O. Skjemstad, D. C. Reicosky, A. R. Wilts, J. A. McGowan. Charcoal carbon in US agricultural soils. *Soil Sci. Soc. Am. J.* **2002**, *66*, 1249.
[5] C. M. Preston, M. W. I. Schmidt. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. *Biogeosciences* **2006**, *3*, 397.
[6] M. W. I. Schmidt. Carbon budget in the black. *Nature* **2004**, *427*, 305.
[7] J. Lehmann. A handful of carbon. *Nature* **2007**, *447*, 143.
[8] G. Cornelissen, O. Gustafsson, T. D. Bucheli, M. T. O. Jonker, A. A. Koelmans, P. M. Van Noort. Extensive sorption of organic compounds to black carbon, coal, and kerogen in sediments and soils: mechanisms and consequences for distribution, bioaccumulation, and biodegradation. *Environ. Sci. Technol.* **2005**, *39*, 6881.
[9] T. H. Nguyen, H. H. Cho, D. L. Poster, W. P. Ball. Evidence for a pore-filling mechanism in the adsorption of aromatic hydrocarbons to a natural wood char. *Environ. Sci. Technol.* **2007**, *41*, 1212.
[10] B. Glaser, J. Lehmann, W. Zech. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoals. A review. *Biol. Fert. Soils* **2002**, *35*, 219.
[11] J. Lehmann, J. Gaunt, M. Rondon. Bio-char sequestration in terrestrial ecosystems: A review. *Mitig. Adapt. Strat. Glob. Chang.* **2006**, *11*, 403.
[12] J. S. Fried, M. S. Torn, E. Mills. The impact of climate change on wildfire severity: a regional forecast for Northern California. *Clim. Chang.* **2004**, *64*, 169.
[13] J. A. González-Pérez, F. J. González-Vila, G. Almendros, H. Knicker. The effect of fire on soil organic matter – a review. *Environ. Intern.* **2004**, *30*, 855.
[14] J. Lehmann. Bio-energy in the black. *Frontiers Ecol. Environ.* **2007**, *5*, 381.
[15] C. E. Brewer, K. Schmidt-Rohr, J. A. Satrio, R. C. Brown. Characterization of biochar from fast pyrolysis and gasification systems. *Env. Progress Sustainable Energy* **2009**, *28*, 386.
[16] J. A. Baldock, R. J. Smernik. Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. *Org. Geochem.* **2002**, *33*, 1093.
[17] C. A. Masiello. New directions in black carbon organic geochemistry. *Mar. Chem.* **2004**, *92*, 201.
[18] S. Bruun, E. S. Jensen, L. S. Jensen. Microbial mineralization and assimilation of PyOM: Dependency on degree of thermal alteration. *Org. Geochem.* **2008**, *39*, 839.

[19] A. V. McBeath, R. J. Smernik. Variation in the degree of aromatic condensation of chars. *Org. Geochem.* **2009**, *40*, 1161.
[20] G. Almendros, H. Knicker, F. J. Gonzalez-Vila. Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C- and ^{15}N-NMR spectroscopy. *Org. Geochem.* **2003**, *34*, 1559.
[21] K. Hammes, R. J. Smernik, J. O. Skjemstad, A. Herzog, U. F. Vogt, M. W. I. Schmidt. Synthesis and characterisation of laboratory-charred grass straw (*Oryza sativa*) and chestnut wood (*Castanea sativa*) as reference materials for PyOM quantification. *Org. Geochem.* **2006**, *37*, 1629.
[22] H. A. Knicker, A. Hilscher, F. J. González-Vila, G. Almendros. A new conceptual model for the structural properties of char produced during vegetation fires. *Org. Geochem.* **2008**, *39*, 935.
[23] J. Kaal, C. Rumpel. Can pyrolysis-GC/MS be used to estimate the degree of thermal alteration of black carbon? *Org. Geochem.* **2009**, *40*, 1179.
[24] J. Kaal, A. M. Cortizas, K. G. J. Nierop. Characterisation of aged charcoal using a coil probe pyrolysis-GC/MS method optimised for black carbon. *J. Anal. Appl. Pyrolysis* **2009**, *85*, 408.
[25] B. R. T. Simoneit, J. J. Schauer, C. G. Nolte, D. R. Oros, V. O. Elias, M. P. Fraser, W. F. Rogge, G. R. Cass. Levoglucosan, a tracer for cellulose in biomass burning and atmospheric. *Atmos. Environ.* **1999**, *33*, 173.
[26] B. Glaser, L. Haumaier, G. Guggenberger, W. Zech. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Org. Geochem.* **1998**, *29*, 811.
[27] S. Brodowski, A. Rodionov, L. Haumaier, B. Glaser, W. Amelung. Revised PyOM assessment using benzene polycarboxylic acids. *Org. Geochem.* **2005**, *36*, 1299.
[28] K. Hammes, M. W. I. Schmidt, R. J. Smernik, L. A. Currie, W. P. Ball, T. H. Nguyen, P. Louchouarn, S. Houel, O. Gustafsson, M. Elmquist, G. Cornelissen, J. O. Skjemstad, C. A. Masiello, J. Song, P. A. Peng, A. Mitra, J. C. Dunn, P. G. Hatcher, W. C. Hockaday, D. M. Smith, C. Hartkopf-Froder, A. Bohmer, A. B. Luer, B. J. Huebert, W. Amelung, S. Brodowski, L. Huang, W. Zhang, P. M. Gschwend, D. X. Flores-Cervantes, C. Largeau, J.-N. Rouzaud, C. Rumpel, G. Guggenberger, K. Kaiser, A. Rodionov, F. J. Gonzalez-Vila, J. A. Gonzalez-Perez, J. M. de la Rosa, D. A. C. Manning, I. E. Lopez-Cape, L. Ding. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. *Global Biogeochem. Cycles* **2007**, *21*, GB3016.
[29] T. Dittmar. The molecular level determination of black carbon in marine dissolved organic matter. *Org. Geochem.* **2008**, *39*, 396.
[30] M. P. W. Schneider, M. Hilf, U. F. Vogt, M. W. I. Schmidt. The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 °C and 1000 °C. *Org. Geochem.* **2010**, *41*, 1082.
[31] X. Dai, T. W. Boutton, B. Glaser, R. J. Ansley, W. Zech. Black carbon in a temperate mixed-grass savanna. *Soil Biol. Biochem.* **2005**, *37*, 1879.
[32] S. Brodowski, S. W. Amelung, L. Haumaier, W. Zech. Black carbon contribution to stable humus in German arable soils. *Geoderma* **2007**, *139*, 220.
[33] G. Guggenberger, A. Rodionov, O. Shibistova, M. Grabe, O. A. Kasansky, H. Fuchs, N. Mikheyeva, G. Zrazhevskaya, H. Flessa. Storage and mobility of black carbon in permafrost soils of the forest tundra ecotone in Northern Siberia. *Global Change Biol.* **2008**, *14*, 1367.
[34] K. Hammes, M. S. Torn, A. G. Lapenas, M. W. I. Schmidt. Centennial black carbon turnover observed in a Russian steppe soil. *Biogeosciences* **2008**, *5*, 1339.
[35] M. P. W. Schneider, R. H. Smittenberg, T. Dittmar, M. W. I. Schmidt. Comparison of gas and liquid chromatography for the determination of benzenepolycarboxylic acids as molecular tracers of black carbon. *Org. Geochem.* **2011**, *42*, 275.
[36] A. R. Zimmerman. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environ. Sci. Technol.* **2010**, *44*, 1295.
[37] B. Glaser, K. H. Knorr. Isotopic evidence for condensed aromatics from non-pyrogenic sources in soils – implications for current methods for quantifying soil black carbon. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 935.
[38] G. Docherty, V. Jones, R. P. Evershed. Practical and theoretical considerations in the gas chromatography/combustion/isotope ratio mass spectrometry δ^{13}C analysis of small polyfunctional molecules. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 730.
[39] M. Krummen, A. W. Hilkert, D. Juchelka, A. Duhr, H. J. Schluter, R. Pesch. A new concept for isotope ratio monitoring liquid chromatography/mass spectrometry. *Rapid Commun. Mass Spectrom.* **2004**, *18*, 2260.
[40] H. T. S. Boschker, T. C. W. Moerdijk-Poortvliet, P. van Breugel, M. Houtekamer, J. J. Middelburg. A versatile method for the stable carbon isotope analysis of carbohydrates by high-performance liquid chromatography/isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2008**, *22*, 3902.
[41] D. A. Abaye, D. J. Morrison, T. Preston. Strong anion exchange liquid chromatographic separation of protein amino acids for the natural ^{13}C-abundance determination by isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* **2011**, *25*, 429.
[42] S. Bode, K. Denef, P. Boeckx. Development and evaluation of a high-performance liquid chromatography/isotope ratio mass spectrometry methodology for δ^{13}C analyses of amino sugars in soil. *Rapid Commun. Mass Spectrom.* **2009**, *23*, 2519.
[43] R. Baziramakenga, R. R. Simard, G. D. Leroux. Determination of organic acids in soil extracts by ion chromatography. *Soil Biol. Biochem.* **1995**, *27*, 349.
[44] Y. C. Lee. Carbohydrate analyses with high-performance anion-exchange chromatography. *J. Chromatogr. A* **1996**, *720*, 137.
[45] M. Waksundzka-Hanjos. Chromatographic separation of aromatic carboxylic acids. *J. Chromatogr. B* **1998**, *717*, 93.
[46] J. A. Bird, M. S. Torn. Fine roots versus needles: A comparison of ^{13}C and ^{15}N dynamics in a ponderosa pine forest soil. *Biogeochemistry* **2006**, *79*, 361.
[47] S. Abiven, A. Heim, M. W. I. Schmidt. Lignin content and chemical characteristics in maize and wheat vary between plant organs and growth stages: consequences for assessing lignin dynamics in soil. *Plant Soil* **2011**, *343*, 369.
[48] C. M. Gough, C. S. Vogel, K. H. Harrold, K. George, P. S. Curtis. The legacy of harvest and fire on ecosystem carbon storage in a north temperate forest. *Global Change Biol.* **2007**, *13*, 1935.
[49] M. W. I. Schmidt, J. O. Skjemstad, E. Gehrt, I. Kogel-Knabner. Charred organic carbon in German chernozemic soils. *Eur. J. Soil Sci.* **1999**, *50*, 351.
[50] J. Cerar, C. Podlipnik. Relationships between aqueous acidities of benzene polycarboxylic acids and computed surface-electrostatic potentials and charges. *Acta Chim. Slov.* **2008**, *55*, 999.
[51] M. P. Ricci, D. A. Merritt, K. H. Freeman, J. M. Hayes. Acquisition and processing of data for isotope-ratio-monitoring mass spectrometry. *Org. Geochem.* **1994**, *21*, 561.
[52] J.-P. Godin, L.-B. Fay, G. Hopfgartner. Liquid chromatography combined with mass spectrometry for ^{13}C isotopic analysis in life science research. *Mass Spectrom. Rev.* **2007**, *26*, 751.
[53] Finnigan LC IsoLink LC-IRMS Interface Operation Manual, Revision B, Thermo Electron Corp. Bremen, Germany, **2004**.

[54] L. Haumaier. Benzene polycarboxylic acids - a ubiquitous class of compounds in soils. *J. Plant Nutr. Soil Sci.* **2010**, *173*, 727.
[55] A. Heim, M. W. I. Schmidt. Lignin turnover in arable soil and grassland analysed with two different labelling approaches. *Eur. J. Soil Sci.* **2007**, *58*, 599.
[56] M.-F. Dignac, H. Bahri, C. Rumpel, D. P. Rasse, G. Bardoux, J. Balesdent, C. Girardin, C. Chenu, A. Mariotti. Carbon-13 natural abundance as a tool to study the dynamics of lignin monomers in soil: an appraisal at the Closeaux experimental field (France). *Geoderma* **2005**, *128*, 3.
[57] D. Derrien, J. Balesdent, C. Marol, C. Santaella. Measurement of the $^{13}C/^{12}C$ ratio of soil-plant individual sugars by gas chromatography/combustion/isotope-ratio mass spectrometry of silylated derivatives. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 2626.
[58] G. L. B. Wiesenberg, J. Schwarzbauer, M. W. I. Schmidt, L. Schwark. Source and turnover of organic matter in agricultural soils derived from *n*-alkane/*n*-carboxylic acid compositions and C-isotope signatures. *Org. Geochem.* **2004**, *35*, 1371.
[59] H. Bahri, M.-F. Dignac, C. Rumpel, D. P. Rasse, C. Chenu, A. Mariotti. Lignin turnover kinetics in an agricultural soil is monomer specific. *Soil Biol. Biochem.* **2006**, *38*, 1977.
[60] L. A. Ziolkowski, E. R. M. Druffel. Aged black carbon identified in marine dissolved organic carbon. *Geophys. Res. Lett.* **2010**, *37*, L16601.
[61] S. Abiven, P. Hengartner, M. P. W. Schneider, N. Singh, M. W. I. Schmidt. Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal. *Soil Biol. Biochem.* **2011**, *43*, 1615.
[62] T. Dittmar, J. Paeng. A heat-induced molecular signature in marine dissolved organic matter. *Nat. Geosci.* **2009**, *2*, 175.

Manuscript V

Pyrogenic carbon soluble fraction is larger and more aromatic in aged charcoal than in fresh charcoal

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Short Communication

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ABSTRACT

Recent studies show that pyrogenic matter is one of the most stable compounds in the soil but less inert than previously expected. One potential pathway yielding losses from soil is solubilisation of pyrogenic compounds. In batch experiments, we estimated the proportion and molecular composition of soluble (<0.45 μm) and colloidal fractions (0.45–5 μm) extractable from a freshly pyrolysed charcoal and a 10 year old wildfire charcoal. These fractions represented a very small fraction (<2.7 g kg^{-1}) of chars. The benzene polycarboxylic acids (BPCA) pattern indicated that 40–55 times more condensed structures were released from the aged char than from the fresh char. This study shows that the soluble fraction of the char is small, and tends to increase with the residence time in the soil.

1. Introduction

Fire-derived organic compounds, also known as pyrogenic carbon (PyC), are ubiquitous in soils and sediments (Schmidt and Noack, 2000) and riverine and oceanic dissolved organic matter (Kim et al., 2004; Dittmar, 2008; Dittmar and Paeng, 2009). Due to its chemical recalcitrance, PyC has been considered as particularly stable in soil (Skjemstad et al., 1996). However, recent studies have suggested that PyC might disappear from soil profiles faster than previously expected (Hammes et al., 2008; Kuzyakov et al., 2009).

Potential mechanisms for PyC losses from soil could be either biotic or abiotic oxidation (Zimmerman, 2010; Cheng et al., 2006), and/or transport by lixiviation or erosion (Rumpel et al., 2006).

In fact, substantial amounts of PyC have been found in riverine and oceanic dissolved organic matter, using either specific molecular markers techniques (Dittmar, 2008) or ultrahigh resolution mass spectrometry (Kim et al., 2004; Hockaday et al., 2006). Mechanisms of transport from soil to river, however, are yet unknown.

According to estimations based on literature, over 80% of the PyC produced gets incorporated into soil (Preston and Schmidt, 2006). PyC probably becomes soluble as the surface becomes increasingly more oxidised (Lehmann et al., 2005). Circumstantial evidence suggests that PyC can be transported by water through the soil profile. Bird et al. (1999) observed that small PyC particles could be transported into underlying soil horizons. Guggenberger et al. (2008) also found an accumulation of PyC-specific molecular biomarkers in lower layers of permafrost. It is, however, not clear how much of the bulk pyrogenic matter becomes soluble during degradation.

The objectives of this study are to estimate the amounts of the potentially soluble and particulate fractions that can be released from a fresh charcoal and from an oxidised 10 year old charcoal, and identify the molecular marker patterns of the leachate.

2. Materials and methods

The fresh char was pyrolysed at 450 °C for 5 h under N_2 from chestnut wood (*Castanea sativa*) according to Hammes et al. (2006). The aged char was collected from an experimental forest fire where chestnut trees had been burned 10 years ago (Prometheus site, Ticino, Switzerland) (Wüthrich et al., 2002). Charcoal pieces (>5 cm) were collected on the soil surface of the plots and gently cleaned using soft brush (in dry state) to remove soil particles attached to it. These charcoal pieces had been in contact with air and soil and therefore we assumed them to be more oxidised than the fresh char. Both chars were produced from the same feedstock and to comparable temperature (wildfire char temperature of 450 °C according to Turney et al., 2006). To homogenize the samples, both fresh and aged chars were air-dried, ground and sieved through a 1 mm sieve.

In batch experiments similar to Kaiser et al. (2001), we tested different ratios of char mass to water volume and shaking

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durations. The combination which produced the largest concentrations of soluble mass was obtained at the ratio of 8 g bulk dry material to 100 ml deionised water, shaken for 6 h. Each treatment was repeated 6 times.

The soluble and colloidal fractions were collected by vacuum filtration (0.45 and 0.45–5 μm), freeze-dried, weighed and analysed for carbon (C) and nitrogen (N) (Vario EL, Elementar Analysis systems, Hanau, Germany).

The benzene polycarboxylic acids (BPCA) molecular marker method was employed to quantify and characterize the PyC in the soluble and colloidal fraction (Glaser et al., 1998; Brodowski et al., 2005; Schneider et al., 2010). Briefly, samples ($n = 3$) were pretreated with 4 M trifluroacetic acid (4 h, 105 °C), followed by conversion of PyC into BPCA by nitric acid oxidation (8 h, 170 °C). The digested extract was purified using cation exchange resin, freeze-dried and subsequently derivatised and analysed on a gas chromatograph equipped with a flame ionization detector. The acids with 3, 4, 5 and 6 carboxyl functions (B3CA, B4CA, B5CA and B6CA, respectively) were identified, quantified and summed up to represent the amount of pyrogenic molecular markers in the material.

3. Results and discussion

The total mass of soluble and colloidal fractions extractable in optimised batch experiments from both fresh and aged chars was small (<0.3% mass of the initial; Table 1). Under field conditions, these chars probably would release even less PyC. Major et al. (2010) also observed that two years after the input of charcoal to the soil, soluble PyC fluxes represented less than 1% of the annual PyC budget.

The elemental composition shows that the soluble and colloidal fractions were made up of relatively less carbon (<50% C) and more nitrogen (>0.6% N), than the bulk material (>67% C and <0.36% N). The nitrogen concentrations were particularly large for both colloidal and soluble fractions of the aged char (>1.80% N). C to N ratios of both char colloidal and soluble fractions (50 and 55 for fresh char and 15 and 18 for the aged char) were very small. This could either correspond to components which have not been pyrolysed or rich-N pyrolysed compounds which are preferentially soluble. The large N content in the aged char could be also due to microbial biomass that is present on the surface of old charcoal pieces (Hockaday et al., 2007) or sorbed dissolved organic matter accumulated along time.

The BPCA markers produced by both chars (125.7 ± 8.1 and 171.6 ± 6.0 g BPCA-C kg^{-1} OC for fresh and aged char) were in line with previous findings (Hammes et al., 2007). Also, the relative proportions of BPCA markers were similar, with slightly more B3CA in the aged char than in the fresh char.

Although the total mass of soluble and colloidal fractions was similar for both chars, the BPCA markers were 40–55 times more abundant in the soluble and colloidal fractions of aged char. We attribute this difference to the biotic or/and abiotic oxidation of the aged char which could lead to the production of soluble PyC clusters.

Table 1
Mass, C and N content and BPCA content in the soluble and colloidal fractions and in the bulk fresh and aged chars, respectively. Mean values in a column within each type of char followed by the different letters are significantly different at $P < 0.05$ using post-hoc analysis Least Significant Difference.

	Mass g kg^{-1}	C g kg^{-1}	N g kg^{-1}	C:N	ΣBPCA gC kg^{-1} OC
Fresh char					
Bulk	1000 ± 0.0a	667 ± 40a	1.0 ± 0.1b	695a	125.7 ± 8.1a
Soluble	1.5 ± 0.1c	374 ± 19b	7.5 ± 0.7a	50b	1.0 ± 0.1b
Colloidal	2.7 ± 0.2b	365 ± 11b	6.7 ± 0.5a	55b	1.3 ± 0.6b
Aged char					
Bulk	1000 ± 0.0a	669 ± 6a	3.6 ± 0.1b	184a	171.6 ± 6.0a
Soluble	1.4 ± 0.1b	294 ± 9b	19.9 ± 1.8a	15b	41.0 ± 0.7c
Colloidal	1.7 ± 0.1b	319 ± 8b	18.0 ± 0.1a	18b	74.0 ± 1.9b

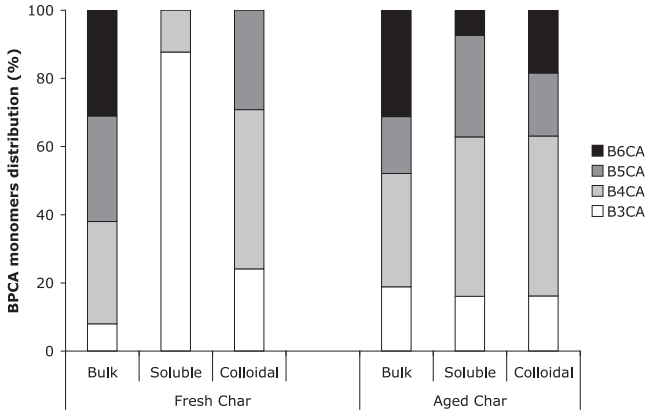

Fig. 1. BPCA monomers distribution (%) of fresh and aged chars, shown for bulk char and soluble and colloidal fractions.

The relative contributions of individual molecular markers reflect the quality of PyC and the size of the aromatic clusters they originate from (Schneider et al., 2010). B3CA and B4CA can be produced from small condensed units of 3 aromatic rings minimum (e.g. retene), while formation of B6CA requires a minimum of five or more condensed rings (Ziolkowski et al., in press). In the soluble and colloidal fractions of the aged char (Fig. 1), the larger content in B6CA indicates that larger molecular clusters became soluble due to oxidation with ageing (Dittmar and Koch, 2006), while such larger clusters are absent in the corresponding fractions from fresh chars. Thus, with residence time in soil, larger and more chemically condensed clusters were released.

The BPCA patterns of the aged char soluble fraction (Fig. 1) are similar to patterns found in dissolved organic matter of Apalachee river bay in Dittmar (2008) or Suwannee river in Ziolkowski and Druffel (2010), i.e. B4CA and B5CA represent the majority of the distribution, while B3CA and B6CA represent 15 and 5% of the total, respectively. If this observation would hold true for PyC breakdown patterns, this would indicate that no major chemical modification happens to the colloidal and dissolved PyC, between ageing in soil and on their way to the river.

The soluble and colloidal fractions extractable from freshly pyrolysed char material are small. With residence time in soil, char releases larger soluble pyrogenic condensed aromatic structures. This soluble and colloidal PyC has a molecular marker pattern similar to soluble PyC found in river DOM.

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References

Bird, M.I., Moyo, C., Veenendaal, E.M., Lloyd, J., Frost, P., 1999. Stability of elemental carbon in a savanna soil. Global Biogeochemical Cycles 13, 923–932.
Brodowski, S., Rodionov, A., Haumaier, L., Glaser, B., Amelung, W., 2005. Revised black carbon assessment using benzene polycarboxylic acids. Organic Geochemistry 36, 1299–1310.

Cheng, C.H., Lehmann, J., Thies, J.E., Burton, S.D., Engelhard, M.H., 2006. Oxidation of black carbon by biotic and abiotic processes. Organic Geochemistry 37, 1477–1488.

Dittmar, T., 2008. The molecular level determination of black carbon in marine dissolved organic matter. Organic Geochemistry 39, 396–407.

Dittmar, T., Koch, B.P., 2006. Thermogenic organic matter dissolved in the abyssal ocean. Marine Chemistry 102, 208–217.

Dittmar, T., Paeng, J., 2009. A heat-induced molecular signature in marine dissolved organic matter. Nature Geoscience 2, 175–179.

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Organic Geochemistry 29, 811–819.

Guggenberger, G., Rodionov, A., Shibistova, O., Grabe, M., Kasanksy, O.A., Fuchs, H., Mikheyeva, N., Zrazhevskaya, G., Flessa, H., 2008. Storage and mobility of black carbon in permafrost soils of the forest tundra ecotone in Northern Siberia. Global Change Biology 14, 1367–1381.

Hammes, K., Schmidt, M.W.I., Smernik, R.J., Currie, L.A., Ball, W.P., Nguyen, T.H., Louchouarn, P., et al., 2007. Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. Global Biogeochemical Cycles 21. doi:10.1029/2006GB002914.

Hammes, K., Smernik, R.J., Skjemstad, J.O., Herzog, A., Vogt, U.F., Schmidt, M.W.I., 2006. Synthesis and characterisation of laboratory-charred grass straw (*Oryza sativa*) and chestnut wood (*Castanea sativa*) as reference materials for black carbon quantification. Organic Geochemistry 37, 1629–1633.

Hammes, K., Torn, M.S., Lapenas, A.G., Schmidt, M.W.I., 2008. Centennial black carbon turnover observed in a Russian steppe soil. Biogeosciences 5, 1339–1350.

Hockaday, W.C., Grannas, A.M., Kim, S., Hatcher, P.G., 2007. The transformation and mobility of charcoal in a fire-impacted watershed. Geochimica et Cosmochimica Acta 71, 3432–3445.

Hockaday, W.C., Grannas, A.M., Kim, S., Hatcher, P.G., 2006. Direct molecular evidence for the degradation and mobility of black carbon in soils from ultra-high-resolution mass spectral analysis of dissolved organic matter from a fire-impacted forest soil. Organic Geochemistry 37, 501–510.

Kaiser, K., Kaupenjohann, M., Zech, W., 2001. Sorption of dissolved organic carbon in soils: effects of soil sample storage, soil-to-solution ratio, and temperature. Geoderma 99, 317–328.

Kim, S., Kaplan, L.A., Benner, R., Hatcher, P.G., 2004. Hydrogen-deficient molecules in natural riverine water samples—evidence for the existence of black carbon in DOM. Marine Chemistry 92, 225–234.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biology and Biochemistry 41, 210–219.

Lehmann, J., Liang, B.Q., Solomon, D., Lerotic, M., Luizao, F., Kinyangi, J., Schafer, T., Wirick, S., Jacobsen, C., 2005. Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy for mapping nano-scale distribution of organic carbon forms in soil: application to black carbon particles. Global Biogeochemical Cycles 19. doi:10.1029/2004GB002435.

Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Global Change Biology 16, 1366–1379.

Preston, C.M., Schmidt, M.W.I., 2006. Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3, 397–420.

Rumpel, C., Chaplot, V., Planchon, O., Bernadou, J., Valentin, C., Mariotti, A., 2006. Preferential erosion of black carbon on steep slopes with slash and burn agriculture. Catena 65, 30–40.

Schmidt, M.W.I., Noack, A.G., 2000. Black carbon in soils and sediments: analysis, distribution, implications, and current challenges. Global Biogeochemical Cycles 14, 777–793.

Schneider, M.P.W., Hilf, M., Vogt, U.F., Schmidt, M.W.I., 2010. The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 °C and 1000 °C. Organic Geochemistry 41, 1082–1088.

Skjemstad, J.O., Clarke, P., Taylor, J.A., Oades, J.M., McClure, S.G., 1996. The chemistry and nature of protected carbon in soil. Australian Journal of Soil Research 34, 251–271.

Turney, C., Wheeler, D., Chivas, A.R., 2006. Carbon isotope fractionation in wood during carbonization. Geochimica et Cosmochimica Acta 70, 960–964.

Wüthrich, C., Schaub, D., Weber, M., Marxer, P., Conedera, M., 2002. Soil respiration and soil microbial biomass after fire in a sweet chestnut forest in southern Switzerland. Catena 48, 201–215.

Zimmerman, A.R., 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environmental Science & Technology 44, 1295–1301.

Ziolkowski, L.A., Chamberlin, A.R., Greaves, J., Druffel, E.R.M. Quantification of black carbon in marine systems using the benzene polycarboxylic acid method: a mechaniistic and yield study. Limnology and Oceanography: Methods 9, in press.

Ziolkowski, L.A., Druffel, E.R.M., 2010. Aged black carbon identified in marine dissolved organic carbon. Geophysical Research Letters 37. doi:10.1029/2010GL043963.

6.3 Elucidate the effect of shift in microbial community structure on soil C stock

Microbial community structure at family rank was observed to change with PyOM amendment within 10 months in the field conditions. However, our ability is limited to interpret the functional consequences of shifts in microbial community in response to added substrate or environmental changes like N deposition. Future research should focus what does this shift indicates in terms of C storage, soil respiration, greenhouse gases efflux or changes in SOM dynamics. Further work would also be required to determine the relative importance of changes in edaphic factors in altering microbial community composition due to PyOM amendments to the soil, and the longer-term effects of PyOM on the microbial community structure and activity.

References

Abiven S, Andreoli R (2010) Charcoal does not change the decomposition rate of mixed litters in a mineral cambisol: a controlled conditions study. *Biology and Fertility of Soils,* **47**, 111-114.

Ågren GI, Mcmurtrie RE, Parton WJ, Pastor J, Shugart HH (1991) State-of-the-Art of models of production decomposition linkages in conifer and grassland ecosystems. *Ecological Applications,* **1**, 118-138.

Allison SD, Lebauer DS, Ofrecio MR, Reyes R, Ta AM, Tran TM (2009) Low levels of nitrogen addition stimulate decomposition by boreal forest fungi. *Soil Biology & Biochemistry,* **41**, 293-302.

Almendros G, Knicker H, Gonzalez-Vila FJ (2003) Rearrangement of carbon and nitrogen forms in peat after progressive thermal oxidation as determined by solid-state ^{13}C- and ^{15}N-NMR spectroscopy. *Organic Geochemistry,* **34**, 1559-1568.

Bailey VL, Fansler SJ, Smith JL, Bolton H (2011) Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. *Soil Biology & Biochemistry,* **43**, 296-301.

Baldock JA, Masiello CA, Gelinas Y, Hedges JI (2004) Cycling and composition of organic matter in terrestrial and marine ecosystems. *Marine Chemistry,* **92**, 39-64.

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally, altered Pinus resinosa (Red Pine) wood. *Organic Geochemistry,* **33**, 1093-1109.

Batjes NH (1996) Total carbon and nitrogen in the soils of the world. *European Journal of Soil Science,* **47**, 151-163.

Bebber DP, Watkinson SC, Boddy L, Darrah PR (2011) Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. *Oecologia,* **167**, 1177-1184.

Beesley L, Marmiroli M (2011) The immobilisation and retention of soluble arsenic, cadmium and zinc by biochar. *Environmental Pollution,* **159**, 474-480.

Bernoux M, Cerri CC, Neill C, De Moraes JFL (1998) The use of stable carbon isotopes for estimating soil organic matter turnover rates. *Geoderma,* **82**, 43-58.

Bird MI, Moyo C, Veenendaal EM, Lloyd J, Frost P (1999) Stability of elemental carbon in a savanna soil. *Global Biogeochemical Cycles,* **13**, 923-932.

Bragazza L, Freeman C, Jones T *et al.* (2006) Atmospheric nitrogen deposition promotes carbon loss from peat bogs. *Proceedings of the National Academy of Sciences,* **103**, 19386-19389.

Brodowski S (2005) Origin, function, and reactivity of black carbon in the arable soil environment. *PhD Thesis, Institut für Bodenkunde, Bonn*, 183.

Brodowski S, John B, Flessa H, Amelung W (2006) Aggregate-occluded black carbon in soil. *European Journal of Soil Science,* **57**, 539-546.

Brodowski S, Rodionov A, Haumaier L, Glaser B, Amelung W (2005) Revised black carbon assessment using benzene polycarboxylic acids. *Organic Geochemistry,* **36**, 1299-1310.

Carcaillet C (2001) Are Holocene wood-charcoal fragments stratified in alpine and subalpine soils? Evidence from the Alps based on AMS C-14 dates. *Holocene,* **11**, 231-242.

Carcaillet C, Richard PJH (2000) Holocene changes in seasonal precipitation highlighted by fire incidence in eastern Canada. *Climate Dynamics,* **16**, 549-559.

Cerar J, Podlipnik C (2008) Relationships Between Aqueous Acidities of Benzene Polycarboxylic Acids and Computed Surface-electrostatic Potentials and Charges. *Acta Chimica Slovenica,* **55**, 999-1008.

Cerli C, Celi L, Kalbitz K, Guggenberger G, Kaiser K (2012) Separation of light and heavy organic matter fractions in soil - Testing for proper density cut-off and dispersion level. *Geoderma,* **170**, 403-416.

Chan KY, Van Zwieten L, Meszaros I, Downie A, Joseph S (2007) Agronomic values of greenwaste biochar as a soil amendment. *Australian Journal of Soil Research,* **45**, 629-634.

Chaplot VaM, Rumpel C, Valentin C (2005) Water erosion impact on soil and carbon redistributions within uplands of Mekong River. *Global Biogeochemical Cycles,* **19**.

Cheng CH, Lehmann J (2009) Ageing of black carbon along a temperature gradient. *Chemosphere,* **75**, 1021-1027.

Cheng CH, Lehmann J, Engelhard MH (2008a) Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochimica Et Cosmochimica Acta,* **72**, 1598-1610.

Cheng CH, Lehmann J, Thies JE, Burton SD (2008b) Stability of black carbon in soils across a climatic gradient. *Journal of Geophysical Research-Biogeosciences,* **113**, -.

Cheng CH, Lehmann J, Thies JE, Burton SD, Engelhard MH (2006) Oxidation of black carbon by biotic and abiotic processes. *Organic Geochemistry,* **37**, 1477-1488.

Chughtai AR, Jassim JA, Peterson JH, Stedman DH, Smith DM (1991) Spectroscopic and solubility characteristics of oxidized soots. . *Aerosol Science and Technology,* **15**, 112-126.

Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. *Proceedings of the National Academy of Sciences of the United States of America,* **103**, 10316-10321.

Clough A, Skjemstad JO (2000) Physical and chemical protection of soil organic carbon in three agricultural soils with different contents of calcium carbonate. *Australian Journal of Soil Research,* **38**, 1005-1016.

Cohen-Ofri I, Weiner L, Boaretto E, Mintz G, Weiner S (2006) Modern and fossil charcoal: aspects of structure and diagenesis. *Journal of Archaeological Science,* **33**, 428-439.

Cusack DF, Chadwick OA, Hockaday WC, Vitousek PM (2012) Mineralogical controls on soil black carbon preservation. *Global Biogeochemical Cycles,* **26**.

Cusack DF, Torn MS, Mcdowell WH, Silver WL (2010) The response of heterotrophic activity and carbon cycling to nitrogen additions and warming in two tropical soils. *Global Change Biology,* **16**, 2555-2572.

Czimczik CI, Masiello CA (2007) Controls on black carbon storage in soils. *Global Biogeochemical Cycles,* **21**.

Czimczik CI, Schmidt MWI, Schulze ED (2005) Effects of increasing fire frequency on black carbon and organic matter in Podzols of Siberian Scots pine forests. *European Journal of Soil Science,* **56**, 417-428.

Dai X, Boutton TW, Glaser B, Ansley RJ, Zech W (2005) Black carbon in a temperate mixed-grass savanna. *Soil Biology & Biochemistry,* **37**, 1879-1881.

Deangelis MM, Wang DG, Hawkins TL (1995) Solid-phase reversible immobilization for the isolation of PCR products. *Nucleic Acids Research,* **23**, 4742-4743.

Denman KL, Brasseur G, Chidthaisong A *et al.* (2007) *Couplings Between Changes in the Climate System and Biogeochemistry. ,* Cambridge, UK and New York, USA, Cambridge University press.

Dittmar T, Paeng J, Gihring TM, Suryaputra IGNA, Huettel M (2012) Discharge of dissolved black carbon from a fire-affected intertidal system. *Limnology and Oceanography,* **57**, 1171-1181.

Eckmeier E, Rosch M, Ehrmann O, Schmidt MWI, Schier W, Gerlach R (2007) Conversion of biomass to charcoal and the carbon mass balance from a slash-and-burn experiment in a temperate deciduous forest. *Holocene,* **17**, 539-542.

Elmquist M, Semiletov I, Guo LD, Gustafsson O (2008) Pan-Arctic patterns in black carbon sources and fluvial discharges deduced from radiocarbon and PAH source apportionment markers in estuarine surface sediments. *Global Biogeochemical Cycles,* **22**.

F.A.O.-U.N.E.S.C.O. (1998) ISSS, ISRIC, FAO. World Reference Base for Soil Resources. FAO, World Soil Resources Reports 84. pp Page, Rome.

Flannigan M, Amiro B, Logan K, Stocks B, Wotton B (2006) Forest Fires and Climate Change in the 21st Century. *Mitigation and Adaptation Strategies for Global Change,* **11**, 847-859.

Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic-Matter. *Biological Reviews of the Cambridge Philosophical Society,* **63**, 433-462.

Forbes MS, Raison RJ, Skjemstad JO (2006) Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. *Science of the Total Environment,* **370**, 190-206.

Fowles M (2007) Black carbon sequestration as an alternative to bioenergy. *Biomass & Bioenergy,* **31**, 426-432.

Galloway JN, Townsend AR, Erisman JW *et al.* (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science,* **320**, 889-892.

Glaser B, Amelung W (2003) Pyrogenic carbon in native grassland soils along a climosequence in North America. *Global Biogeochemical Cycles,* **17**.

Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W (2000) Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Organic Geochemistry,* **31**, 669-678.

Glaser B, Haumaier L, Guggenberger G, Zech W (1998) Black carbon in soils: the use of benzenecarboxylic acids as specific markers. *Organic Geochemistry,* **29**, 811-819.

Glaser B, Haumaier L, Guggenberger G, Zech W (2001) The 'Terra Preta' phenomenon: a model for sustainable agriculture in the humid tropics. *Naturwissenschaften,* **88**, 37-41.

Glaser B, Knorr KH (2008) Isotopic evidence for condensed aromatics from non-pyrogenic sources in soils - implications for current methods for quantifying soil black carbon. *Rapid Communications in Mass Spectrometry,* **22**, 935-942.

Golchin A, Baldock JA, Clarke P, Higashi T, Oades JM (1997) The effects of vegetation and burning on the chemical composition of soil organic matter of a volcanic ash soil as shown by C-13 NMR spectroscopy .2. Density fractions. *Geoderma,* **76**, 175-192.

Goldberg ED (1985) *Black carbon in the environment: properties and distribution,* New York, John Wiley and Sons.

Gouveia SEM, Pessenda LCR (2000) C-14 dating of charcoal in the soil for the study of biological remount of soil matter and of the colluvium in the formation of ferralsols of Sao Paulo State, southern Brazil. *Comptes Rendus De L Academie Des Sciences Serie Ii Fascicule a-Sciences De La Terre Et Des Planetes,* **330**, 133-138.

Gustafsson O, Gschwend PM (1998) The flux of black carbon to surface sediments on the New England continental shelf. *Geochimica Et Cosmochimica Acta,* **62**, 465-472.

Hakkenberg R, Churkina G, Rodeghiero M, Borner A, Steinhof A, Cescatti A (2008) Temperature sensitivity of the turnover times of soil organic matter in forests. *Ecological Applications,* **18**, 119-131.

Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry,* **35**, 823-830.

Hammes K, Schmidt MWI (2009) Changes of biochar in soil. In: *Biochar for environmental Management.* (eds Lehmann J, Joseph S) pp Page. London, Earthscan.

Hammes K, Schmidt MWI, Smernik RJ *et al.* (2007) Comparison of quantification methods to measure fire-derived (black/elemental) carbon in soils and sediments using reference materials from soil, water, sediment and the atmosphere. *Global Biogeochemical Cycles,* **21**, GB3016.

Hammes K, Smernik RJ, Skjemstad JO, Herzog A, Vogt UF, Schmidt MWI (2006) Synthesis and characterisation of laboratory-charred grass straw (Oryza saliva) and chestnut wood (Castanea sativa) as reference materials for black carbon quantification. *Organic Geochemistry,* **37**, 1629-1633.

Hammes K, Torn MS, Lapenas AG, Schmidt MWI (2008) Centennial black carbon turnover observed in a Russian steppe soil. *Biogeosciences,* **5**, 1339-1350.

Hansen J, Nazarenko L (2004) Soot climate forcing via snow and ice albedos. *Proceedings of the National Academy of Sciences of the United States of America,* **101**, 423-428.

Haumaier L (2010) Benzene polycarboxylic acids-A ubiquitous class of compounds in soils. *Journal of Plant Nutrition and Soil Science,* **173**, 727-736.

Haumaier L, Zech W (1995) Black Carbon - Possible Source of Highly Aromatic Components of Soil Humic Acids. *Organic Geochemistry,* **23**, 191-196.

Hedges JI, Keil RG (1995) Sedimentary Organic-Matter Preservation - an Assessment and Speculative Synthesis. *Marine Chemistry,* **49**, 81-115.

Hilscher A, Heister K, Siewert C, Knicker H (2009) Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. *Organic Geochemistry,* **40**, 332-342.

Hilscher A, Knicker H (2011a) Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. *Soil Biology & Biochemistry,* **43**, 261-270.

Hilscher A, Knicker H (2011b) Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. *Organic Geochemistry,* **42**, 42-54.

Hofmann A (2009) Lignin dynamics in arable soils as determined by 13C natural abundance. Unpublished Dr.sc.nat. University of Zurich, Zurich.

Janssens IA, Dieleman W, Luyssaert S *et al.* (2010) Reduction of forest soil respiration in response to nitrogen deposition. *Nature Geoscience,* **3**, 315-322.

Jansson C, Wullschleger SD, Kalluri UC, Tuskan GA (2010) Phytosequestration: Carbon Biosequestration by Plants and the Prospects of Genetic Engineering. *Bioscience,* **60**, 685-696.

Jones DL, Murphy DV, Khalid M, Ahmad W, Edwards-Jones G, Deluca TH (2011) Short-term biochar-induced increase in soil CO2 release is both biotically and abiotically mediated. *Soil Biology & Biochemistry,* **43**, 1723-1731.

Keiluweit M, Nico PS, Johnson MG, Kleber M (2010) Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar). *Environmental Science & Technology,* **44**, 1247-1253.

Khodadad CLM, Zimmerman AR, Green SJ, Uthandi S, Foster JS (2011) Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. *Soil Biology & Biochemistry,* **43**, 385-392.

Kloeti P, Keller HM, Guecheva M (1989) Effects of forest canopy on throughfall precipitation chemistry. In: *Proceedings of a Symposium held during the Third Scientific Assembly of the International Association of Hydrological Sciences.* pp Page, Baltimore, Maryland, Atmospheric Deposition.

Knicker H (2011) Pyrogenic Organic Matter in Soil: Its Origin and Occurrence, its Chemistry and Survival in Soil Environments. *Quaternary International,* **243**, 251-263.

Knicker H, Almendros G, Gonzalez-Vila FJ, Gonzalez-Perez JA, Polvillo O (2006) Characteristic alterations of quantity and quality of soil organic matter caused by forest fires in continental Mediterranean ecosystems: a solid-state C-13 NMR study. *European Journal of Soil Science,* **57**, 558-569.

Knicker H, Almendros G, Gonzalezvila FJ, Martin F, Ludemann HD (1996) C-13- and N-15-NMR spectroscopic examination of the transformation of organic nitrogen in plant biomass during thermal treatment. *Soil Biology & Biochemistry,* **28**, 1053-1060.

Knicker H, Gonzalez-Vila FJ, Polvillo O, Gonzalez JA, Almendros G (2005a) Fire-induced transformation of C- and N-forms in different organic soil fractions from a Dystric Cambisol under a Mediterranean pine forest (Pinus pinaster). *Soil Biology & Biochemistry,* **37**, 701-718.

Knicker H, Totsche KU, Almendros G, Gonzalez-Vila FJ (2005b) Condensation degree of burnt peat and plant residues and the reliability of solid-state VACP MAS C-13 NMR spectra obtained from pyrogenic humic material. *Organic Geochemistry,* **36**, 1359-1377.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: A meta-analysis. *Ecology,* **86**, 3252-3257.

Kolb SE, Fermanich KJ, Dornbush ME (2009) Effect of Charcoal Quantity on Microbial Biomass and Activity in Temperate Soils. *Soil Science Society of America Journal,* **73**, 1173-1181.

Krull ES, Swanston CW, Skjemstad JO, Mcgowan JA (2006) Importance of charcoal in determining the age and chemistry of organic carbon in surface soils. *Journal of Geophysical Research-Biogeosciences,* **111**.

Kuhlbusch TaJ (1998) Black carbon and the carbon cycle. *Science,* **280**, 1903-1904.

Kuzyakov Y, Subbotina I, Chen HQ, Bogomolova I, Xu XL (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. *Soil Biology & Biochemistry,* **41**, 210-219.

Laird DA (2008) The charcoal vision: A win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agronomy Journal,* **100**, 178-181.

Laird DA, Chappell MA, Martens DA, Wershaw RL, Thompson M (2008) Distinguishing black carbon from biogenic humic substances in soil clay fractions. *Geoderma,* **143**, 115-122.

Lal R (2004) Soil carbon sequestration impacts on global climate change and food security. *Science,* **304**, 1623-1627.

Lehmann J, Da Silva JP, Steiner C, Nehls T, Zech W, Glaser B (2003) Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. *Plant and Soil,* **249**, 343-357.

Lehmann J, Gaunt J, Rondon M (2006) Bio-Char sequestration in terrestrial ecosystems- A review. *Mitigation and Adaptation Strategies for Global Change,* **11**, 403-427.

Lehmann J, Liang BQ, Solomon D *et al.* (2005) Near-edge X-ray absorption fine structure (NEXAFS) spectroscopy for mapping nano-scale distribution of organic carbon forms in soil: Application to black carbon particles. *Global Biogeochemical Cycles,* **19**.

Liang B, Lehmann J, Solomon D *et al.* (2006) Black Carbon increases cation exchange capacity in soils. *Soil Science Society of America Journal,* **70**, 1719-1730.

Liang B, Lehmann J, Solomon D *et al.* (2008) Stability of biomass-derived black carbon in soils. *Geochimica Et Cosmochimica Acta,* **72**, 6069-6078.

Lohmann R, Bollinger K, Cantwell M, Feichter J, Fischer-Bruns I, Zabel M (2009) Fluxes of soot black carbon to South Atlantic sediments. *Global Biogeochemical Cycles,* **23**.

Mack MC, Schuur EaG, Bret-Harte MS, Shaver GR, Chapin FS (2004) Ecosystem carbon storage in arctic tundra reduced by long-term nutrient fertilization. *Nature,* **431**, 440-443.

Major J, Lehmann J, Rondon M, Goodale C (2009) Fate of soil-applied black carbon: downward migration, leaching and soil respiration. *Global Change Biology,* **16**, 1366-1379.

Marris E (2006) Putting the carbon back: Black is the new green. *Nature,* **442**, 624-626.

Masiello CA (2004) New directions in black carbon organic geochemistry. *Marine Chemistry,* **92**, 201-213.

Masiello CA, Druffel ERM (1998) Black carbon in deep-sea sediments. *Science,* **280**, 1911-1913.

Masiello CA, Druffel ERM (2001) Carbon isotope geochemistry of the Santa Clara River. *Global Biogeochemical Cycles,* **15**, 407-416.

Masiello CA, Druffel ERM (2003) Organic and black carbon C-13 and C-14 through the Santa Monica Basin sediment oxic-anoxic transition. *Geophysical Research Letters,* **30**.

Mccoll JG, Powers RF (1998) Decomposition of small diameter woody debris of red fir determined by nuclear magnetic resonance. *Communications in Soil Science and Plant Analysis,* **29**, 2691-2704.

Mcconnell JR, Edwards R, Kok GL *et al.* (2007) 20th-century industrial black carbon emissions altered arctic climate forcing. *Science,* **317**, 1381-1384.

Micks P, Downs MR, Magill AH, Nadelhoffer KJ, Aber JD (2004) Decomposing litter as a sink for N-15-enriched additions to an oak forest and a red pine plantation. *Forest Ecology and Management,* **196**, 71-87.

Middelburg JJ, Nieuwenhuize J, Van Breugel P (1999) Black carbon in marine sediments. *Marine Chemistry,* **65**, 245-252.

Mitra S, Bianchi TS, Mckee BA, Sutula M (2002) Black carbon from the Mississippi River: Quantities, sources, and potential implications for the global carbon cycle. *Environmental Science & Technology,* **36**, 2296-2302.

Moritz MA, Parisien M-A, Batllori E, Krawchuk MA, Dorn JV, Ganz DJ, Hayhole K (2012) Climate change and disruptions to global fire activity. *Ecosphere,* **3**, 1-22.

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003) Microbial diversity and soil functions. *European Journal of Soil Science,* **54**, 655-670.

Nguyen BT, Lehmann J (2009) Black carbon decomposition under varying water regimes. *Organic Geochemistry,* **40**, 846-853.

Nguyen BT, Lehmann J, Hockaday WC, Joseph S, Masiello CA (2010) Temperature Sensitivity of Black Carbon Decomposition and Oxidation. *Environmental Science & Technology,* **44**, 3324-3331.

Nguyen BT, Lehmann J, Kinyangi J, Smernik R, Riha SJ, Engelhard MH (2008) Long-term black carbon dynamics in cultivated soil. *Biogeochemistry,* **89**, 295-308.

Norby RJ (1998) Nitrogen deposition: a component of global change analyses. *New Phytologist,* **139**, 189-200.

Pind A, Freeman C, Lock MA (1994) Enzymatic Degradation of Phenolic Materials in Peatlands - Measurement of Phenol Oxidase Activity. *Plant and Soil,* **159**, 227-231.

Potter MC (1908) Bacteria as agents in the oxidation of amorphous carbon. *Proceedings of the Royal Society of London Series B-Containing Papers of a Biological Character,* **80**, 239-259.

Preston CM, Schmidt MWI (2006) Black (pyrogenic) carbon: a synthesis of current knowledge and uncertainties with special consideration of boreal regions. *Biogeosciences,* **3**, 397-420.

Ramanathan V, Carmichael G (2008) Global and regional climate changes due to black carbon. *Nature Geoscience,* **1**, 221-227.

Ronaghi M (2001) Pyrosequencing sheds light on DNA sequencing. *Genome Research,* **11**, 3-11.

Ronaghi M, Uhlen M, Nyren P (1998) A sequencing method based on real-time pyrophosphate. *Science,* **281**, 363-+.

Ruehr NK, Buchmann N (2010) Soil respiration fluxes in a temperate mixed forest: seasonality and temperature sensitivities differ among microbial and root-rhizosphere respiration. *Tree Physiology,* **30**, 165-176.

Rumpel C, Alexis M, Chabbi A, Chaplot V, Rasse DP, Valentin C, Mariotti A (2006a) Black carbon contribution to soil organic matter composition in tropical sloping land under slash and burn agriculture. *Geoderma,* **130**, 35-46.

Rumpel C, Chaplot V, Planchon O, Bernadou J, Valentin C, Mariotti A (2006b) Preferential erosion of black carbon on steep slopes with slash and burn agriculture. *Catena,* **65**, 30-40.

Saiya-Cork KR, Sinsabaugh RL, Zak DR (2002) The effects of long term nitrogen deposition on extracellular enzyme activity in an Acer saccharum forest soil. *Soil Biology & Biochemistry,* **34**, 1309-1315.

Sanchez-Garcia L, Cato I, Gustafsson O (2012) The sequestration sink of soot black carbon in the Northern European Shelf sediments. *Global Biogeochemical Cycles,* **26**.

Santos F, Torn MS, Bird JA (2012) Biological degradation of pyrogenic organic matter in temperate forest soils. *Soil Biology & Biochemistry,* **51**, 115-124.

Schmidt MWI, Noack AG (2000) Black carbon in soils and sediments: Analysis, distribution, implications, and current challenges. *Global Biogeochemical Cycles,* **14**, 777-793.

Schmidt MWI, Skjemstad JO, Gehrt E, Kogel-Knabner I (1999) Charred organic carbon in German chernozemic soils. *European Journal of Soil Science,* **50**, 351-365.

Schmidt MWI, Torn MS, Abiven S *et al.* (2011) Persistence of soil organic matter as an ecosystem property. *Nature,* **478**, 49-56.

Schneider MPW (2011) Assessment of a Molecular Marker Method to Determine the Pyrogenic Carbon Component in Charcoals and Soils. Unpublished PhD University of Zurich, Zurich, 82 pp.

Schneider MPW, Hilf M, Vogt UF, Schmidt MWI (2010) The benzene polycarboxylic acid (BPCA) pattern of wood pyrolyzed between 200 degrees C and 1000 degrees C. *Organic Geochemistry,* **41**, 1082-1088.

Schneider MPW, Lehmann J, Schmidt MWI (2011) Charcoal quality does not change over a century in a tropical agro-ecosystem. *Soil Biology & Biochemistry,* **43**, 1992-1994.

Seiler W, Crutzen PJ (1980) Estimates of Gross and Net Fluxes of Carbon between the Biosphere and the Atmosphere from Biomass Burning. *Climatic Change,* **2**, 207-247.

Shafizadeh F, Sekiguchi Y (1983) Development of Aromaticity in Cellulosic Chars. *Carbon,* **21**, 511-516.

Shen SM, Pruden G, Jenkinson DS (1984) Mineralization and Immobilization of Nitrogen in Fumigated Soil and the Measurement of Microbial Biomass Nitrogen. *Soil Biology & Biochemistry,* **16**, 437-444.

Shneour EA (1966) Oxidation of graphitic carbon in certain soils. . *Science,* **151**, 991-992.

Singh N, Abiven S, Torn MS, Schmidt MWI (2012) Fire-derived organic carbon in soil turns over on a centennial scale. *Biogeosciences,* **9**, 2847-2857.

Sinsabaugh RL, Gallo ME, Lauber C, Waldrop MP, Zak DR (2005) Extracellular enzyme activities and soil organic matter dynamics for northern hardwood forests receiving simulated nitrogen deposition. *Biogeochemistry,* **75**, 201-215.

Skjemstad JO, Dalal RC, Janik LJ, Mcgowan JA (2001) Changes in chemical nature of soil organic carbon in Vertisols under wheat in south-eastern Queensland. *Australian Journal of Soil Research,* **39**, 343-359.

Skjemstad JO, Taylor JA, Janik LJ, Marvanek SP (1999) Soil organic carbon dynamics under long-term sugarcane monoculture. *Australian Journal of Soil Research,* **37**, 151-164.

Sombroek WG, Nachtergaele FO, Hebel A (1993) Amounts, Dynamics and Sequestering of Carbon in Tropical and Subtropical Soils. *Ambio,* **22**, 417-426.

Sombroek WG, Ruivo MDL, Fearnside PM, Glaser B, J L (2003) Amazonian Dark Earths as carbon stores and sinks. In: *Amazonian Dark earths: Origins, Properties, Management.* (ed J. L) pp Page. Dordrecht, Kluwer Academic Publishers.

Spokas KA (2010) Review of the stability of biochar in soils: predictability of O:C molar ratios. *Carbon Management,* **1**, 289-303.

Steinbeiss S, Gleixner G, Antonietti M (2009) Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology & Biochemistry,* **41**, 1301-1310.

Swanston C, Homann PS, Caldwell BA, Myrold DD, Ganio L, Sollins P (2004) Long-term effects of elevated nitrogen on forest soil organic matter stability. *Biogeochemistry,* **70**, 227-250.

Thery-Parisot I, Chabal L, Chrzavzez J (2010) Anthracology and taphonomy, from wood gathering to charcoal analysis. A review of the taphonomic processes modifying charcoal assemblages, in archaeological contexts. *Palaeogeography Palaeoclimatology Palaeoecology,* **291**, 142-153.

Topoliantz S, Ponge JF (2003) Burrowing activity of the geophagous earthworm Pontoscolex corethrurus (Oligochaeta : Glossoscolecidae) in the presence of charcoal. *Applied Soil Ecology,* **23**, 267-271.

Townsend AR, Braswell BH, Holland EA, Penner JE (1996) Spatial and temporal patterns in terrestrial carbon storage due to deposition of fossil fuel nitrogen. *Ecological Applications,* **6**, 806-814.

Turunen J, Roulet NT, Moore TR, Richard PJH (2004) Nitrogen deposition and increased carbon accumulation in ombrotrophic peatlands in Eastern Canada. *Global Biogeochemical Cycles,* **18**.

Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. *Soil Biology & Biochemistry,* **19**, 703-707.

Vasilyeva NA, Abiven S, Milanovskiy EY, Hilf M, Rizhkov OV, Schmidt MWI (2011) Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem. *Soil Biology & Biochemistry,* **43**, 1985-1988.

Von Lützow M, Kögel-Knabner I, Ekschmitt K, Matzner E, Guggenberger G, Marschner B, Flessa H (2006) Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *European Journal of Soil Science,* **57**, 426-445.

Wal A, Boer W, Smant W, Veen J (2007) Initial decay of woody fragments in soil is influenced by size, vertical position, nitrogen availability and soil origin. *Plant and Soil,* **301**, 189-201.

Waldrop MP, Zak DR, Sinsabaugh RL, Gallo M, Lauber C (2004) Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications,* **14**, 1172-1177.

Wardle DA, Nilsson MC, Zackrisson O (2008) Fire-derived charcoal causes loss of forest humus. *Science,* **320**, 629-629.

Woolf D, Lehmann J (2012) Modelling the long-term response to positive and negative priming of soil organic carbon by black carbon. *Biogeochemistry,* **111**, 83-95.

Wu J, Joergensen RG, Pommerening B, Chaussod R, Brookes PC (1990) Measurement of Soil Microbial Biomass C by Fumigation Extraction - an Automated Procedure. *Soil Biology & Biochemistry,* **22**, 1167-1169.

Yang YN, Sheng GY (2003) Enhanced pesticide sorption by soils containing particulate matter from crop residue burns. *Environmental Science & Technology,* **37**, 3635-3639.

Yano Y, Lajtha K, Sollins P, Caldwell BA (2005) Chemistry and dynamics of dissolved organic matter in a temperate coniferous forest on Andic soils: Effects of litter quality. *Ecosystems,* **8**, 286-300.

Yarnes C, Santos F, Singh N, Abiven S, Schmidt MWI, Bird JA (2011) Stable isotopic analysis of pyrogenic organic matter in soils by liquid chromatography-isotope-ratio mass spectrometry of benzene polycarboxylic acids. *Rapid Communications in Mass Spectrometry,* **25**, 3723-3731.

Zackrisson O, Nilsson MC, Wardle DA (1996) Key ecological function of charcoal from wildfire in the Boreal forest. *Oikos,* **77**, 10-19.

Zalamea M, Gonzalez G, Ping CL, Michaelson G (2007) Soil organic matter dynamics under decaying wood in a subtropical wet forest: effect of tree species and decay stage. *Plant and Soil,* **296**, 173-185.

Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science & Technology,* **44**, 1295-1301.

Zimmerman AR, Gao B, Ahn MY (2011) Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology & Biochemistry,* **43**, 1169-1179.

Ziolkowski LA, Druffel ERM (2009) The feasibility of isolation and detection of fullerenes and carbon nanotubes using the benzene polycarboxylic acid method. *Marine Pollution Bulletin,* **59**, 213-218.

Ziolkowski LA, Druffel ERM (2010) Aged black carbon identified in marine dissolved organic carbon. *Geophysical Research Letters,* **37**.

Part C – Appendix

Global PyOM-C stock data

Table A1: Global PyOM-C stock data from previous studies

Sl no.	Site description	Climate	Soil type	Latitude	Longitude	PyOM (% of total C)	Depth (cm)	Methodology	Reference
1	Congo, West Africa: 15 km north-east of Pointe Noire (300-310 cm depth)	Tropical	sandy ferralic soil classified as ‘‘ferrallitique psammitique’’ or ferralic arenosol,	-4.78	11.85	<5	300-310	Step-wise separation and collection of non hydrolysable residue, elemental analysis	(Poirier *et al.*, 2002)
2	Cultivated soil (from slash and burn forest), South Nandi, Western Kenya (n = 9)	Tropical, MAT = 19˚C and MAP = 2000 mm	Humic Nitosols (FAO/UNESCO) OR typic Palehumults (USDA)	0.08	34.98	10–29	0-90	Nuclear Magnetic Resonance (NMR)	(Nguyen *et al.*, 2008)
3	Sandy soil from suburban area in Guangzhou City, China (n = 3)	Humid subtropical	Brownish/yellowish sandy soil, riversediment (depth 0-15 cm) and pond sediment	23.13	113.27	35.9	0-10	Step-wise fractionation into different C fractions, NMR	(Song *et al.*, 2002)
4	A permafrost-free glacial-fluvial sand plain, eastern edge of the west Siberian lowland, 40 km west of the river Yenisey (n = 5)	Continental, 17.3˚C in July to -22.9˚C in January	Dystrustepts	60.67	89.13	4.5	0-10	benzenepolycarboxylic acids (BPCA)	(Czimczik *et al.*, 2003)
5	Volcanic ash soils, Japan (n= 24)	Sub Tropical	Volcanic ash soils	42.98 42.07 42.07 39.68 39.73 39.20 39.20 36.38	141.57 143.15 143.15 140.98 141.08 141.12 141.12 139.73	9.34 15.84 9.17 11.58 6.37 26.16 21.39 7.64	24–35 19–35 35–45 27–41 32–49 23–35 35–63 32–46	Isolation of charred plant fragments using sodium polytungstate, digested with hydrochloric acid:hydrofluric acid (HCl:HF)	(Shindo *et al.*, 2004)

				36.57	139.75	7.27	23–45		
				36.03	140.07	7.3	30–48		
				35.22	138.62	19.61	15–27		
				35.22	138.62	3.79	30–45		
				35.22	138.62	4.13	45–65		
				34.72	137.85	3.37	39–62		
				35.55	134.82	5.46	15–48		
				35.55	134.82	20	24–51		
				35.45	133.77	7.296	24–34		
				35.22	132.67	32.73	22–47		
				34.80	132.85	6.16	32–42		
				32.72	130.67	15.55	30–50		
				31.99	130.95	21.2	22–66		
				31.72	131.07	10.28	30–37		
				31.72	131.07	7.86	31–44		
				31.72	131.07	5.42	44–70		
6	Eastern edge of the West Siberian Plain, 40 km west of the river Yenisey, Russia (n= 10, soils differing in fire regimes)	Continental 18˚C in July and -24˚C in January, MAP = 560 mm	Gleyic, Cambic, and Haplic Podzols	60.76 60.74	89.40 89.44	0.02 0.02	0-25 0-25	BPCA	(Czimczik *et al.*, 2005)
7	Luang Prabang Province, Laos (n = 16)	Average annual rainfall is 1403 mm and MAT =25˚C	Dytrochrepts, Alfisols, and Inceptisols	19.88 19.88 19.88 19.88 19.88 19.88 19.88 19.88 19.88 19.88 19.88	102.13 102.13 102.13 102.13 102.13 102.13 102.13 102.13 102.13 102.13 102.13	5.50 5.50 3.70 4.10 7.30 3.40 5.90 3.50 2.90 6.80 4.50	0-40 0-20 20-35 35-60 0-30 60-80 0-25 25-55 55-75 0-5 5-15	Analyzed on HF treated samples by oxidation resistant elemental carbon (OREC) using potassium dichromate at 60˚C	(Rumpel *et al.*, 2006a)

				19.88	102.13	4.60	15-40		
				19.88	102.13	6.80	0-10		
				19.88	102.13	4.20	10-25		
				19.88	102.13	4.00	25-50		
8	Zonal Steppe soils of Russia (n = 6) from six sites.	MAP = 715 mm, MAT = 4.1˚C	Greyzems, Phaeozems, Chernozems, and Kastanozems	54.00	37.50	3.5	0-10	BPCA	(Rodionov *et al.*, 2006)
		MAP = 573 mm, MAT = 5.3˚C		51.75	36.17	10.1	0-10		
		MAP = 450 mm, MAT = 5.5˚C		50.00	44.50	12.1	0-10		
		MAP = 300 mm, MAT = 6˚C		47.33	44.50	7.7	0-10		
		MAP = 300 mm, MAT = 7˚C		54.00	37.50	8.6	50-60		
		MAP = 325 mm, MAT=8˚C		51.75	36.17	17.2	50-60		
9	South Australia, Queensland (various soils) (n= 3)		Agrixeroll	-34.83	138.88	13.12	0-10	Photo-oxidation and ^{13}C Nuclear Magnetic Resonance (NMR)	(Skjemstad *et al.*, 1996)
			Haploudoll	-27.58	152.55	5.06	0-5		
			Kandiustox	-27.57	151.95	6.24	0-5		
10	Australia, 8 surface soils from Queensland (n= 5) and South Australia (n=3)		Haploudoll	-34.83	138.88	0.9	0-5	Photo-oxidation and ^{13}C NMR of <53 µm soil fractions	(Skjemstad *et al.*, 1999)
			Kandiustox	-27.57	151.95	15	0-5		
			Pellustert	-27.57	151.95	30	0-10		
			Agrixeroll	-27.58	152.55	26	0-10		
			Rhodoxeralf	-34.92	138.60	21	0-10		
			Chromustert	-27.57	151.95	24	0-5		
			Chromustert	-26.75	150.63	4	0-10		
			Natrixeralf	-33.13	136.42	10	0-10		
11	Waco soil and Langlands-Logie soil, Australia		Pellustert	-26.66	151.30	32.98	0-10	High energy UV photo-oxidation along with solid-state ^{13}C NMR spectroscopy	(Skjemstad *et al.*, 2001)
			Chromustert	-26.54	150.83	11.11	0-10		
12	Australia, 2 long-term agriculture sites (i) Brigalow Research Station	Semi-arid, subtropical	Two clays, a duplex soil and a red brown earth	-24.83	149.78	25.02	0-30	UV photo-oxidation along with solid-state ^{13}C NMR spectroscopy	(Skjemstad *et al.*, 2004)

	in Queensland with continuous wheat and some sorghum was established after clearing land under brigalow (*Acacia harpophylla*) and continued for 18 years (ii) Tarlee, South Australia, was established on existing agricultural land								
13	Loess overlying Wuermian fluvic gravel (30 km south of Munich)	1400 mm y^{-1} of rain on average	Humic Cambisol	48.42 48.42	11.57 11.57	15 30	20-45 45-55	Photo-oxidation followed by ^{13}C CPMAS (Cross polarization-Magic angle spinning) NMR	(Schmidt *et al.*, 1999)
	Baltic coast close to Grossenbrode	Temperate	Humic-stagnic Luvisol	54.47 54.47 54.47	11.13 11.13 11.13	4 15 8	0-30 30-40 40-60		
	North of Halle/Saale in Seeben, Germany		Haplic Phaeozem	52.88 52.88	11.03 11.03	9 15	0-20 20-50		
	Hildesheim-Braunschweig, Northern Germany		Haplic Chernozem	52.15 52.15 52.27 52.27	9.95 9.95 10.52 10.52	21 45 10 10	0-20 20-60 0-20 20-45		
14	Two sites, Saint-Michel-de-Maurienne and Aussois (10 km apart in a valley from northern Alps)	Southern Mediterranean to northern continental		45.25 45.25 44.70 44.60 44.35	6.50 6.75 6.97 6.87 6.80	0.1-20	0-20	Charcoal separated by floatation and manually (>0.4 mm) and charcoal concentration expressed as dried charcoal mass to dried soil mass <2 cm in diameter	(Carcaillet & Talon, 2001)
15	Chernozemic horizons (n=15) originating from six sites: Diedenhofen,	Temperate	Humic cambisol Humic-stagnic Luvisol Haplic Phaeozem	47.87 54.38 51.53	11.58 11.13 12.00	26.52 14.17 13.72	45-55 30-40 20-50	High energy UV photooxidation and Electron energy loss	(Schmid *et al.*, 2002)

	Grossenbrode, Seeben, Sossmar, Harsum, and Adenstedt		Haplic Chernozem Haplic chernozem Haplic greyzem	52.20 52.20 52.20	9.85 9.90 9.93	26.89 42.70 4.69	20-45 20-60 20-45	spectroscopy	
16	Two pits and one ditch of a Neolithic settlement in Murr, approximately 50 km northeast of Munich in southern Germany.	Temperate	Cambisols and Luvisols	48.97 48.97 48.95 48.95	9.27 9.27 9.27 9.27	3.39 20.75 7.10 35.85	80-115 115-130 70-100 120-130	High energy UV photo-oxidation along with solid-state ^{13}C NMR spectroscopy and BPCA	(Schmid *et al.*, 2002)
17	Swiss soil monitoring network (NABO) sample (n = 23)	Temperate	Gleyic cambisol Gleyic cambisol Haplic luvisol Dystric podzoluvisol Chromic luvisol Dystric gleysol Eutric cambisol Gleyic cambisol Dystric podzoluvisol Haplic podzol Folic histosol Haplic podzol Calcic cambisol Eutric cambisol Dystric cambisol Eutric cambisol Eutric cambisol Calcic cambisol Haplic podzol Gleyic cambisol Mollic fluvisol Eutric cambisol Gleyic cambisol	47.48 47.39 47.53 47.22 47.40 46.25 46.98 47.45 46.72 46.80 47.32 46.58 49.89 47.05 47.05 47.50 47.20 47.07 46.01 46.15 46.82 46.27 47.07	8.91 8.57 7.57 7.79 8.18 6.28 6.61 8.75 6.53 9.83 9.57 8.50 7.28 7.47 9.45 8.75 7.43 7.27 8.86 8.98 8.65 7.87 8.43	3.8 2.0 4.8 1.0 3.2 1.6 1.9 1.1 1.3 0.7 2.5 1.7 2.6 3.7 1.4 5.3 2.3 33.6 1.4 6.6 2.0 2.4 6.4	0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10	CTO-375	(Bucheli *et al.*, 2004)
18	The IFA, Borris A,	Temperate		55.97	8.65	4.7	0-10	The organic carbon	(van den

	Kettering, and Askov soils from Austria, Denmark, England, and Denmark, respectively (n = 4).			59.39 55.47	-0.72 9.09	12.8 19.7	0-10 0-10	content after heating the soil at 375˚C for 24 h	Heuvel *et al.*, 2005)
19	German arable soils from the Static Fertilization Experiment established in 1902 in Bad Lauchstädt, Germany; soil profile is located about 30 km to the West of Bad Lauchstädt and was sampled at 7 depths and soils from the long-term field trial "Julius Kühn" in Halle (Saale), Germany	Temperate MAP = 480 mm; MAT = 8.7-9.0˚C	Haplic Chernoze Haplic Phaeozem Haplic Phaeozem	51.37 51.48 48.36	11.83 11.97 13.20	13.3 13.1 23.3 11.5 13.2 16.7 25.2 12.1 12.9 19.8 50.0	0-43 43-60 60-120 0-37 37-54 54-78 78-96 0-40 40-83 83-100 100-128		(Brodowski *et al.*, 2007)
20	La Vall de Gallinera, in Alacant province (E Spain)	Mediterranean MAT = 17.4˚C, MAP = 825 mm.	Leptosols over limestone and calcaric Cambisols over marls	38.82	-0.22	8.62	0-5	Low-temperature dichromate oxidation	(Rovira *et al.*, 2009)
21	Soils were from long-term research plots in widely different agricultural areas (n = 5), North America		frigid Aquic Glos- sudalfs mesic Aquic Argiudoll thermic Udic Haplusterts frigid Typic Calciaquoll mesic Typic Haploxeroll	45.76 41.53 31.08 45.72 45.72	-93.07 -88.12 -97.36 -94.08 -118.00	9.68 23.00 20.60 32.93 34.95	0-20 0-20 0-20 0-20 0-20	High energy ultraviolet photo-oxidation and Solid-State ^{13}C CP-MAS NMR	(Skjemstad *et al.*, 2002)
22	Soils with varying clay content (170-350 g kg^{-1}) along temperature and precipitation transects across the native North American prairies	0.9˚C & 456 mm 1.6˚C & 343 mm 3.2˚C & 380 mm 6.1˚C & 565 mm 5.0˚C & 419 mm 6.1˚C & 300 mm 7.2˚C & 400 mm 10.8˚C & 375 mm	mixed Typic Cryoborolls mixed Typic Cryoborolls mixed Argic Cryoborolls mixed Pachic Udic Haploborolls mixed Typic Haploborolls mixed Aridic Argiborolls mesic Ustic Haplargids mesic Ardic Paleustolls	52.92 52.19 50.17 45.35 46.50 48.33 44.50 40.10	-105.80 -106.17 -107.50 -95.55 -100.54 -109.41 -105.51 -103.13	11.57 7.39 6.51 14.17 8.64 3.75 12.35 4.28	0-10 0-10 0-10 0-10 0-10 0-10 0-10 0-10	BPCA	(Glaser & Amelung, 2003)

		11.6˚C & 666 mm	Typic Argiustolls	40.26	-99.22	7.68	0-10		
		12.2˚C & 573 mm	Entic Haplustolls	38.53	-99.20	5.04	0-10		
		10.9˚C & 792 mm	mesic Typic Argiudolls	40.48	-96.42	8.55	0-10		
		12.4˚C & 791 mm	mesic Pachic Argiudolls	39.11	-96.35	17.56	0-10		
		14.2˚C & 1000 mm	thermic Mollic Albaqualfs	37.20	-95.16	8.38	0-10		
		17.1˚C & 466 mm	thermic Aridic Paleustalfs	32.15	-101.28	6.50	0-10		
		20˚C & 1030 mm	thermic Uderthic Paleustolls	29.42	-96.33	8.38	0-10		
		20.3˚C & 1308 mm	thermic Typic Argiaquolls	30.05	-94.06	9.48	0-10		
		22.2˚C & 700 mm	hyperthermic Typic Paleustolls	27.45	-98.04	11.20	0-10		
		23.4˚C & 440 mm as MAT &MAP	hyperthermic Ustollic Calciorthids	27.57	-98.54	4.60	0-10		
23	Surface horizon of a Black Chernozem, referred to as the Canadian Chernozem, was collected from the Ellerslie Research Station south of the University of Alberta, Edmonton, Canada		Chernozem	53.53	-113.50	22.3	0-10	Chemically oxidized with sodium hypochlorite followed by solid-state ^{13}C NMR	(Simpson & Hatcher, 2004)
24	Two sites, one formed on loamy sand (Meota Association) and the other on silt loam (Blaine Lake Association) from Saskatchewan, Canada	Humid continental to semi arid steppe	Black chernozem	50.07 52.83	-108.77 -106.88	25- 65	Ah and Ab horizons	UV oxidation using the 1.2 Kw lamp as the energy source	(Ponomarenko & Anderson, 2001)
25	The Wurtsmith Air Force Base 2A topsoil from the National Center of Integrated Bioremediation Research and Development (University			44.45 39.13 41.33 38.67	-83.38 -75.46 -72.92 -121.40	17.4 3.7 7.4 12.1	0-10 0-10 0-10 0-10	The organic carbon content after heating the soil or sediment at 375˚C for 24 h	(van den Heuvel *et al.*, 2005)

	of Michigan, Ann Arbor, MI, USA), the Dover dark gray silt loam (DGSL) soil was from Dover Air Force Base (Wilmington, DE, USA), Cheshire soil from Lockwood Farm, Connecticut Agricultural Experiment Station (Hamden, CT, USA) and the McClellan Air Force Base S-1 soil from the National Test Site at McClellan Air Force Base (Sacramento, CA, USA)								
26	The planalto of the Brazilian Amazon basin, where Terra Preta soils occur in small areas embedded in a landscape of infertile Acrisols, Nitisols, Lixisols, and Ferralsols (Terra Preta soils near Manaus and 500 km north of Manaus near Santarem)	Tropical	Terra Preta soils	-3.08 -3.22 -3.25 -2.92 -3.60 -3.08 -3.22 -3.25 -2.92 -3.60	-60.37 -60.40 -60.42 -54.05 -54.93 -60.37 -60.40 -60.42 -54.05 -54.93	13.23 12.17 17.01 18.72 31.00 14.28 24.10 27.91 34.49 17.72	0-10 0-10 0-10 0-10 0-10 30-40 30-41 30-42 30-43 30-44		(Glaser *et al.*, 2000)
27	Australian National soil Archive (NSA) (n = 58)	Different climates	Soils represent all major soil types, climatic regions and vegetation zones in Australia	-40.96 -38.60 -37.02 -37.02 -36.01 -36.14 -33.30 -33.30 -31.79	144.89 141.39 140.38 139.92 146.38 145.02 138.70 138.70 116.09	mean is 32.9 (ranging between 7 to 67)	0-100		Lehmann et al., 2008

				-32.32 -26.37 -26.81 -25.55 -21.54 -21.29 -19.85 -18.66	150.78 151.15 153.10 152.78 146.96 139.82 146.02 145.45				
	Queensland transect (St. George in the south to Duaringa in the north)	Mean annual rainfall and temperatures from 496 mm and 20° C in the south, to 311 mm and 22° C in the north		-23 to -28	145-146	Mean is 31.5; (range between 0 to 81.7)			
	Darwin transect (Darwin to Wauchope)	Mean annual rainfall decreasing from 1560 mm to 270 mm north to south and pronounced seasonality, but relatively constant mean annual temperature of 25°C		-12.45 to -20.63	130.833 to 134.216	Mean is 13.6; (ranging between 0-58.6)			

References

Brodowski S, Amelung W, Haumaier L, Zech W (2007) Black carbon contribution to stable humus in German arable soils. *Geoderma,* **139**, 220-228.

Bucheli TD, Blum F, Desaules A, Gustafsson O (2004) Polycyclic aromatic hydrocarbons, black carbon, and molecular markers in soils of Switzerland. *Chemosphere,* **56**, 1061-1076.

Carcaillet C, Talon B (2001) Soil carbon sequestration by Holocene fires inferred from soil charcoal in the dry French Alps. *Arctic Antarctic and Alpine Research,* **33**, 282-288.

Czimczik CI, Preston CM, Schmidt MWI, Schulze ED (2003) How surface fire in Siberian Scots pine forests affects soil organic carbon in the forest floor: Stocks, molecular structure, and conversion to black carbon (charcoal). *Global Biogeochemical Cycles,* **17**.

Czimczik CI, Schmidt MWI, Schulze ED (2005) Effects of increasing fire frequency on black carbon and organic matter in Podzols of Siberian Scots pine forests. *European Journal of Soil Science,* **56**, 417-428.

Glaser B, Amelung W (2003) Pyrogenic carbon in native grassland soils along a climosequence in North America. *Global Biogeochemical Cycles,* **17**.

Glaser B, Balashov E, Haumaier L, Guggenberger G, Zech W (2000) Black carbon in density fractions of anthropogenic soils of the Brazilian Amazon region. *Organic Geochemistry,* **31**, 669-678.

Nguyen BT, Lehmann J, Kinyangi J, Smernik R, Riha SJ, Engelhard MH (2008) Long-term black carbon dynamics in cultivated soil. *Biogeochemistry,* **89**, 295-308.

Poirier N, Derenne S, Balesdent J, Rouzaud JN, Mariotti A, Largeau C (2002) Abundance and composition of the refractory organic fraction of an ancient, tropical soil (Pointe Noire, Congo). *Organic Geochemistry,* **33**, 383-391.

Ponomarenko EV, Anderson DW (2001) Importance of charred organic matter in Black Chernozem soils of Saskatchewan. *Canadian Journal of Soil Science,* **81**, 285-297.

Rodionov A, Amelung W, Haumaier L, Urusevskaja I, Zech W (2006) Black carbon in the Zonal steppe soils of Russia. *Journal of Plant Nutrition and Soil Science,* **169**, 363-369.

Rovira P, Duguy B, Vallejo VR (2009) Black carbon in wildfire-affected shrubland Mediterranean soils. *Journal of Plant Nutrition and Soil Science,* **172**, 43-52.

Rumpel C, Alexis M, Chabbi A, Chaplot V, Rasse DP, Valentin C, Mariotti A (2006) Black carbon contribution to soil organic matter composition in tropical sloping land under slash and burn agriculture. *Geoderma,* **130**, 35-46.

Schmid EM, Skjemstad JO, Glaser B, Knicker H, Kogel-Knabner I (2002) Detection of charred organic matter in soils from a Neolithic settlement in Southern Bavaria, Germany. *Geoderma,* **107**, 71-91.

Schmidt MWI, Skjemstad JO, Gehrt E, Kogel-Knabner I (1999) Charred organic carbon in German chernozemic soils. *European Journal of Soil Science,* **50**, 351-365.

Shindo H, Honna T, Yamamoto S, Honma H (2004) Contribution of charred plant fragments to soil organic carbon in Japanese volcanic ash soils containing black humic acids. *Organic Geochemistry,* **35**, 235-241.
Simpson MJ, Hatcher PG (2004) Overestimates of black carbon in soils and sediments. *Naturwissenschaften,* **91**, 436-440.
Skjemstad JO, Clarke P, Taylor JA, Oades JM, Mcclure SG (1996) The chemistry and nature of protected carbon in soil. *Australian Journal of Soil Research,* **34**, 251-271.
Skjemstad JO, Dalal RC, Janik LJ, Mcgowan JA (2001) Changes in chemical nature of soil organic carbon in Vertisols under wheat in south-eastern Queensland. *Australian Journal of Soil Research,* **39**, 343-359.
Skjemstad JO, Reicosky DC, Wilts AR, Mcgowan JA (2002) Charcoal carbon in US agricultural soils. *Soil Science Society of America Journal,* **66**, 1249-1255.
Skjemstad JO, Spouncer LR, Cowie B, Swift RS (2004) Calibration of the Rothamsted organic carbon turnover model (RothC ver. 26.3), using measurable soil organic carbon pools. *Australian Journal of Soil Research,* **42**, 79-88.
Skjemstad JO, Taylor JA, Janik LJ, Marvanek SP (1999) Soil organic carbon dynamics under long-term sugarcane monoculture. *Australian Journal of Soil Research,* **37**, 151-164.
Song JZ, Peng PA, Huang WL (2002) Black carbon and kerogen in soils and sediments. 1. Quantification and characterization. *Environmental Science & Technology,* **36**, 3960-3967.
Van Den Heuvel H, Le Couriaut T, Mcmullen BM, Lozac' H F, Van Noort P (2005) Maximum capacities for adsorption of phenanthrene in the slowly and very slowly desorbing domains in nineteen soils and sediments. *Environmental Toxicology and Chemistry,* **24**, 830-835.

Supplementary material, Manuscript I

Methodology

1. Average turnover time: We calculated turnover times based on two data point for each study, the initial stock of PyC and final PyC remaining at the end of the experiment, using Eq. (1). Most studies had only these two data points and intermediate points that were reported in few studies (Brodowski, 2005, Hamer *et al.*, 2004, Kuzyakov *et al.*, 2009) were not included for consistency. The turnover time for compiled data set was then estimated by averaging individual turnover times. We observed that turnover time for PyC ranges from <1 to 750 years and average value of 88 y (Supplementary Figure 2).

$$C_t = C_0 e^{-kt} \quad (1)$$

where C_t is the remaining stock after time t (y), C_0 is the initial stock of PyC (t =0), k is the decay rate (y^{-1}) and turnover time τ (y) =$1/k$.

2. For model fit turnover time: The compiled data set (n = 54) was used to generate time series decrease in stock of PyC (with initial stock at time t = 0 being 100 % and the last point of each study correspond to remaining stock at time t in the time series) and two models used in this study were fitted to it using non linear regression.

a) One-pool model was fitted using constrained non-linear regression using chi-square minimization in IBM SPSS statistics software package for Mac.

b) Two-pool model was fit to the compiled data set using constrained non-linear parameter estimation procedures in IBM SPSS statistics software package for Mac. The curve fitting values were iterative and required initial starting values. To avoid errors due to convergence to local minima of residual sum of squares (RSS), we adopted convergence criteria used by Updegraff (Updegraff *et al.*, 1995), where final parameter estimates were accepted only if equations converged to the same values

given starting values up to 50% above and below them. The explained variance for two-pool model is given in Supplement Table 3.

Statistical analysis

Non-parametric test for comparison between factors were carried using Wilcoxon rank sum test. A three way ANOVA was done on the data set using R software to test the interaction between the variables (Supplement Table 4). Therefore, the imbalanced design of the grouped data (Supplement Table 5) does not introduce any significant error in the interpretation.

Supplement Table A2: Studies that calculated the mean residence time (and/or half life) of PyC using modelling approach. Most studies assume two-pool model approach with a fast mineralizable pool and a slow mineralizable pool.

References	Duration of experiment	Type of experiment	Type of char	Model used to predict MRT	Parameters	Assumptions	MRT calculated
(Baldock & Smernik 2002)	120 days	Incubation	Sap wood, *Pinus resinosa* (charred at <200° and 200-350º C)	No model used			Not calculated
(Hamer *et al.*, 2004)	60 days	Incubation	Maize (at 350° C) Rye (at 350º) and wood at (at 800º C)	**Two-pool decay model**	1. Finely ground PyC material 2. Water holding capacity (WHC) was adjusted to 60% 3. Samples were incubated at 60°C. 4. pH value of maize/sand, rye/sand and wood/sand were 8.0, 6.7, and 6.5, respectively.	Twice as much PyC was mineralized in first 26 days compared to next 30 days suggested, one fast degradable pool and other slow degradable pool.	39 years for charred straw residues and 76 years for charred wood
(Brodowski, 2005)	2 years	Incubation	*Zea mays* (maize) and *Secale cereale* (rye straw) at 350° C	**One-pool decay model** $X_t = X_e + (100\text{-}Xe) \exp(-kt)$ where X_t is PyC concentration at incubation time t , X_e calculated end PyC concentration, k is rate constant($year^{-1}$)	1. Finely ground PyC 2. WHC = 70% 3. incubated at 20°C in dark	All PyC is degradable, $X_t = 100 \exp(-kt)$	Turnover time of 8 years
(Hammes *et al.*,	100 years	Field	Wildfire in a	**One pool, donor**	1. Mean annual	1) PyC is homogeneous	Maximum

2008)			steppe land cover	**controlled model** $\tau = -t/[\ln(f-b)/(f-1)]$ where τ = turnover times(in years); t = time between samplings (years); f+ ratio of modern PyC flux to historic input flux; b = fraction of original PyC stock remaining	temperature (MAT) between 1989-1998 was 6.6°C while between 1893-1950 was 5.5°C. 2. Total annual rainfall between 1989-1998 iwas 507.7 mm and between 1893-1950 was 438.5 mm.	with respect to turnover 2) loss of PyC from soil is a first order decay process 3) after 1900 sampling , PyC inputs decreased in accord with the decrease in regional fire frequency	turnover time to be between 444-541 years and minimum to be between 212-262 years.
(Nguyen *et al.*, 2008)	100 years	Field	Slash and burn of native forest	Used **One pool model** with three parameters $f = Y_0 + ae^{-bt}$ $f = Y_0 + a(1-e^{-bt})$ where f = PyC content at time t (year); Y_0 = PyC content at time zero; a = constant, b = reaction rate constant	1. MAT = 19°C 2. MAP = 2000 mm 3. deep dark reddish soil with friable clay and thick humic topsoil with 45-49% clay, 15-25% silt and 26-40% sand. 4. PyC size particle ranged from 5 to 90 m. 5. Stocks were calculated based on PyC content and bulk density in the top 0.1 m	1. Erosion losses presumed to be low since flat landscape positions were selected to minimize lateral soil export 2. Long term losses by erosion or vertical transport were low since PyC stocks did not decrease beyond 20 years.	8.3 years of MRT (a rate change of 0.12 $year^{-1}$ of total PyC)
(Liang *et al.*, 2008)	532 days (1.5 years)	Incubation	High-PyC containing Anthrosols soils	**Two-pool decay model** $X_t = X_1 (1-e^{-k1t}) + X_2 (1-e^{-k2t})$ where X_t =mineralizable C; X1 = size of the stable C	Turnover time of total SOC (calculated as the ratio of soil C and CO_2-C loss over 532 days at 30°C incubation temperature)	Two pool consisting of 1) a large stable pool with a slow turnover rate comprising PyC and /or stable SOM 2) a smaller and easily	Turnover time was between 44-52 years in BC rich soils as compared

				pool; k1 and k2 = mineralization rates of the labile and stable pools, respectively; and t = time of incubation (days)		mineralizable C pool of higher turnover rate	to 9-20 years in adjacent soils.
(Cheng *et al.*, 2008a)	130 years	Field	Hardwood (chestnut, hickory, oak and sugar maple) produced similarly as wildfire	Not used	n/a	n/a	Not calculated
(Lehmann *et al.*, 2008)	100 year	Modeling approach with different scenarios	Savannah region wildfire PyC	Using single exponential function for scenario 4 i.e. both biomass consumption by fire and the formation of PyC is considered and PyC disappears over time. PyC mineralization was calculated with a **first order decay** to CO_2	1. Equilibrium conditions were established for PyC and non-PyC pools for an average of each soil set 2. MAP= 887 mm and 738 mm, MAT for both is 27°C 3. Clay content is 13% and 21 %, respectively	1.60-90% biomass burned 2.Conversion of burnt biomass to PyC to be between 1-4.5% 3. Belowground C input from grass vegetation was not altered as a result of burning.	Between 718 to 9259 years Applying MRT of 1300 yr could SOC and PyC modelled equilibrium matched experimental observations
(Cheng *et al.*, 2008b)	177 days for PyC conataing soil 50 days for isolated PyC particles	Incubation	PyC samples were collected from the remnants of historic charcoal blast furnances, which were deposited during 1870s. PyC were produced from woods of chestnut,	For PyC, a **one pool decay model** was used to fit OC mineralization kinetics $OC_{cum}(t) = OC_0 (1-\exp(-kt)$ where $OC_{cum}(t)$ = cumulative mineralized OC at time t; OC_0 = the amount of "potential"	1. WHC = 60% 2. Incubated at 30°C 3. 130 year old PyC samples collected from soil.		Mean half life for potential mineralized OC was around 19 days (varied between 10-31 days)

			hickory, oak and sugar maple	mineralizable OC (mg g^{-1} PyC-C); k = decomposition rate constant for potential C mineralization (day^{-1})			
(Cheng & Lehmann, 2009)	1 year	Incubation	Wood logs of white oak and red oak prepared similarly as furnance making charcoal	Not used	1. Aerobic incubation 2. At temperature, -22°C, 4°C, 30°C and 70°C. 3. In water medium	n/a	Not calculated
(Hilscher *et al.*, 2009)	48 days	Incubation	Rye grass (*Lolium perenne*), Pine wood (*Pinus sylvestris*) charred 350° C under oxic condition for 1 minute and 4 minutes	**Two pool decay model**	Controlled aerobic conditions	Used mean mineralization rate of last 10 days when the mineralization rate showed no decline	14 and 19 years for charred rye grass residues and upto 56 years for pine wood chars.
(Kuzyakov *et al.*, 2009)	1089 days (3.9 years)	Incubation	^{14}C labeled *Lolium perenne* charred for 13 h at 400° C	Decomposition of PyC was estimated based on $^{14}CO_2$ efflux and mean **decomposition rate was calculated based on loss of PyC**	1. Soils were incubated at 70% water holding capacity 2. Incubated at 20°C 3. Two types of soil, one with low amount of organic C	Biological activity of a loamy soil in the field is about 10% of that under optimal conditions based on biological active time approach for loamy soil.	Observed MRT = 200 years and based on the assumption of field condition, MRT = 2000 years
(Liang *et al.*, 2010)	532 days	Incubation	PyC rich Anthrosols with distinct ^{13}C isotopic composition using	**Two-pool decay model** $X_t = X_1 (1-e^{-k1t}) + X_2 (1-e^{-k2t})$ where X_t =mineralizable C;		Two pool consisting of 1) a large stable pool with a slow turnover rate comprising PyC and /or stable SOM	Does not calculate MRT for PyC

			the C4 plant was added to originally C3 dominated soil	X1 = size of the stable C pool; k1 and k2 = mineralization rates of the labile and stable pools, respectively; and t = time of incubation (days)		2) a smaller and easily mineralizable C pool of higher turnover rate	
(Major *et al.*, 2009a)	2 years	Field	Mango tree (*Mangifera indica L.*) charred between 400-600° C	**Two-pool decay model**	1. at 26°C mean annual temperature (MAT) 2. PyC was ground and < 0.9 mm 3. PyC applied at the onset of the dry season and incorporated to soil under native savanna vegetation that was never tilled or cropped.First order decay to CO_2		MRT of 600 years [When normalized to MRT = 10°C (from 26°C using a Q_{10} of 3.4) the resulting MRT = 3264 years]
(Zimmerman, 2010)	1 year	Incubation	*Quercus laurifolia* (living wood oak); *Pinus taeda* (pine); *Juniperus virginiana* (cedar); *Guibourtia demusei* (tropical hardwood); *Tripsacum dactyloides* (mixed stems and blades of gamma grass); Sugarcane baggase charred at 250° C under oxic condition and at	$C_{lost} = C_0 - C_t = [C_0 e^b/(m+1)] \times t^{m+1}$ $C_{1/2} = [(m+1)/2e^b]^{\{1/(m+1)\}}$ Where C0 = initial c amount at time t0; Ct = final C amount at final time t, m is slope and b is intercept	1) Coarse size fraction (0.25-2 mm) 2) Direct relationship was observed between the logarithmically transformed experimental degradation rate (k in units of $year^{-1}$) and time (in units of years)	1) Biphasic composition consisting of a more labile volatile component of relatively lower C and higher O content and a non-volatile, high C and low O material 2) time degradation rate relationship is maintained in future	Half life varies between 260-840 years for 250°C, 370-23,800 years for 400°C, 930-12,800 years for 525°C, 15,600- 2.0 x 10^7 years for 650°C.

			400° C, 524° C and 650° C under N_2					
(Hilscher & Knicker 2011b)	28 months	Incubation	Rye grass (*Lolium perenne*), Pine wood (*Pinus sylvestris*) charred 350° C under oxic condition for 1 minute and 4 minutes	**Two-pool decay model** $y = a \cdot e^{(-k1 \cdot t1)} + b \cdot e^{(-k2 \cdot t2)}$ where a = fast decomposable OM pool; b = slowly decomposable OM pool; k_1 and k_2 are turnover constant rate ($year^{-1}$) at respective time t	1. The water content of the soil samples was adjusted to ca. 60% of the maximum water holding capacity 2. Incubated at 30 °C in the dark under aerobic conditions.	1. linear regression model resulted in lower R^2 in the range 0.42–0.82, which supports the idea that the PyOM is composed of C pools with different decomposition kinetics.	The calculated $t_{1/2}$ implies mean residence times between 26 and 31 years for the more stable Pool B.

Supplement Table A3: Studies used in the meta-analysis to calculate the turnover time of PyC in terrestrial systems using mono-exponential decay model.

Reference	Experimental set up	Matrix	Soil type	Climate/ moisture/ temperature	Type of substrate	Pyrolysis temp	Study duration (year)	% of the initial PyC	Individual k ($year^{-1}$)	Individual MRT, first order decay (year)
(Baldock & Smernik, 2002)	Incubation	Sand	Sand (packed at 1.6 bulk density	At 25º C and volumetric water content of 0.29 cm^3 water cm^{-3} soil	Wood	150°C	0.33	87	0.4260	2.35
					Wood	>200°C	0.33	98	0.0618	16.18
(Hamer *et al.*, 2004)	Incubation	Sand	Sand + 1ml inoculum + 0.5ml nutrient solution	At 20º C and 60% water holding capacity (WHC)	Maize (Grass)	350°C	0.16	99.22	0.0479	20.87
					Rye (Grass)	350°C	0.16	99.28	0.0442	22.62
					Wood	800°C	0.16	99.74	0.0159	62.79

(Brodowski, 2005)	Incubation	Soil sand Soil	Top soil; Ap horizon; 0-25 cm: Haplic Phaeozem	At 20º C and 70% WHC	Maize (Grass) Maize (Grass) Rye (Grass)	350°C 350°C 350°C	2.00 2.00 2.00	78.73 48.04 77.91	0.1196 0.3666 0.1248	8.36 2.73 8.01
(Cheng *et al.*, 2008a)	Field	Soil	Soil	Incubated at 30º C and70º C	Oak wood	450-600°C	130.00	77.64	0.0019	513.66
(Bruun *et al.*, 2008)	Incubation	Soil	Sandy loam	25º C	14C labelled Barley root (Hordeum vulgare)	225°C 300°C 375°C	0.08 0.08 0.08	98.1 98.6 91.8	0.2494 0.1833 1.1123	4.01 5.46 0.90
(Major *et al.*, 2009a)	Field	Soil	Isohyperthermic kaolinitic Typic Haplustox sandy clay loam	MAT = 26º C MAP = 2200 mm (95% of precipitation falls between April and December)	prunings of mango tree	600°C	2.00	97.8	0.0111	89.91
(Hilscher *et al.*, 2009)	Incubation	Soil	Bw horizon of Cambisol under Spruce	Incubated at 30º C	Lolium perenne (Grass) Lolium perenne (Grass) Pinus sylvestris (Wood) Pinus sylvestris (Wood	350°C, 1 minutes 350°C, 4 minutes 350°C, 1 minutes 350°C, 4 minutes	0.13 0.13 0.13 0.13	96.9 97.5 99.34 99.54	0.2408 0.1936 0.0506 0.0353	4.15 5.17 19.75 28.36

(Kuzyakov *et al.*, 2009)	Incubation	Soil	Ap horizon of a loamy Haplic Luvisol	Incubated at 20º C and 70% WHC	shoot litter of L perenne	400°C	3.23	95.5	0.0143	70.17
(Zimmerman, 2010)	Incubation	Sand	Quartz Sand	Incubated in the dark at 32 °C	Oak (Wood)					
					Pine (wood)	250°C	1.00	97.8	0.0222	44.95
					Cedar (Wood)	250°C	1.00	97.07	0.0297	33.63
					Bubinga (wood)	250°C	1.00	98.72	0.0129	77.62
					Gamma grass	250°C	1.00	98.6	0.0141	70.93
					Sugar Cane	250°C	1.00	98.8	0.0121	82.83
					(Grass)	250°C	1.00	98.3	0.0171	58.32
					Oak (Wood)					
					Pine (wood)	450°C	1.00	97.93	0.0209	47.81
					Cedar (Wood)	450°C	1.00	98.88	0.0113	88.78
					Bubinga	450°C	1.00	98.95	0.0106	94.74
					(Wood)	450°C	1.00	99.11	0.0089	111.86
					Gamma grass					
					Sugar Cane	450°C	1.00	96.98	0.0307	32.61
					(Grass)	450°C	1.00	98.17	0.0185	54.14
					Oak (Wood)					
					Pine (Wood)	525°C	1.00	99.22	0.0078	127.70
					Cedar (Wood)	525°C	1.00	99.16	0.0084	118.55
					Gamma grass	525°C	1.00	99.07	0.0093	107.03
					Sugar Cane	525°C	1.00	98.43	0.0158	63.19
					(Grass)	525°C	1.00	98.83	0.0118	84.97
					Oak (Wood)					
					Pine (wood)	650°C	1.00	99.15	0.0085	117.15
					Cedar (Wood)	650°C	1.00	99.09	0.0091	109.39
					Bubinga	650°C	1.00	99.45	0.0055	181.32
					(Wood)	650°C	1.00	99.38	0.0062	160.79
					Gamma grass					
					Sugar Cane	650°C	1.00	98.75	0.0126	79.50
					(Grass)	650°C	1.00	99.4	0.0060	166.17

(Nocentini *et al.*, 2010)	Incubation	Sand	Sand	Incubated at 20º C and 50% WHC	Pine needles Pine wood	350°C 350°C	0.08 0.08	99.53 99.43	0.0612 0.0743	16.33 13.46
(Singh *et al.*, 2010)	Incubation	Soil	top Cambisol soil	Incubated at 25º C and 60% WHC	Pinus ponderosa (wood)	450°C	0.15	99.86	0.0091	109.81
(Bird *et al.*, 1999)	Field	Soil	Coarse sand derived from gneissic granite bedrock	Sub-humid MAT =17.7º C MAP = 630 mm		Wildfire	100.00	50	0.0069	144.27
(Hammes *et al.*, 2008)	Field	Soil	Chernozem soil	MAT = 6.6º C (1989-1998) and 5.3ºC (1893-1950); MAP = 507.7 mm (1989-1998) and 438.5 mm (1893-1950)	Grassland	Wildfire	100.00	75	0.0029	347.61
(Nguyen *et al.*, 2008)	Field	Soil	Humic Nitosols (FAO/UNESCO)	Tropical MAT = 19º C MAP = 2000 mm	Forest	Wildfire	100.00	68.52	0.0038	264.51
(Cheng *et al.*, 2008a)	Incubation	Soil	Subsurface soil for incubation	Aged charcoal from areas with MAT ranging between 3.9º C to 17.2º C and	Hardwood (chestnut, hickory, oak and sugar maple)	450-600°C	130.00	77.7	0.0019	515.23

				MAP between 940 mm to 1500 mm						
(Vasilyeva *et al.*, 2010)	Field	Soil	Chernozem	MAT = +5.5 ˚C MAP = 600 mm/year	Grassland	Wildfire	55.00	93	0.0013	757.88
(Bruun *et al.*, 2011a)	Incubation	Soil	Sandy Loam (Typic Hapludalf)	Incubated at room temperature (20-23º C) at constant water content (30%)	Wheat straw	475 500 525 550 575	0.32 0.32 0.32 0.32 0.32	88.10 92.10 94.60 96.00 96.90	0.4022 0.2613 0.1762 0.1296 0.1000	2.49 3.83 5.67 7.72 10.00

Supplement Table A4: ANOVA for two-pool model

	Sum of Squares (SS)	df	Mean squares (MS)
Regression	473627.92	3	157875.97
Residual	3897.936	51	76.43
Uncorrected Total	477525.86	54	
Corrected Total	7010.12	53	

R squared = 1 – (Residual Sum of Squares)/(Corrected Sum of Squares) = 0.444

Supplement Table A5: Three-way ANOVA for interactions between variables

	Df	**Sum sq**	**Mean sq**	**F value**	**Pr(>F)**
Matrix	2	24838	12419	11.7306	0.0001418 ***
Temperature of pyrolysis	1	35051	35051	33.1074	1.992e-06 ***
Initial biomass	2	3915	1958	1.8490	0.1733280
Medium: Temperature of pyrolysis	1	240	240	0.2263	0.6374453
Medium: initial biomass	1	34	34	0.0321	0.8588546
Temperature of pyrolysis: initial biomass	1	3440	3440	3.2488	0.0806158
Residuals	33	34937	1059		

Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

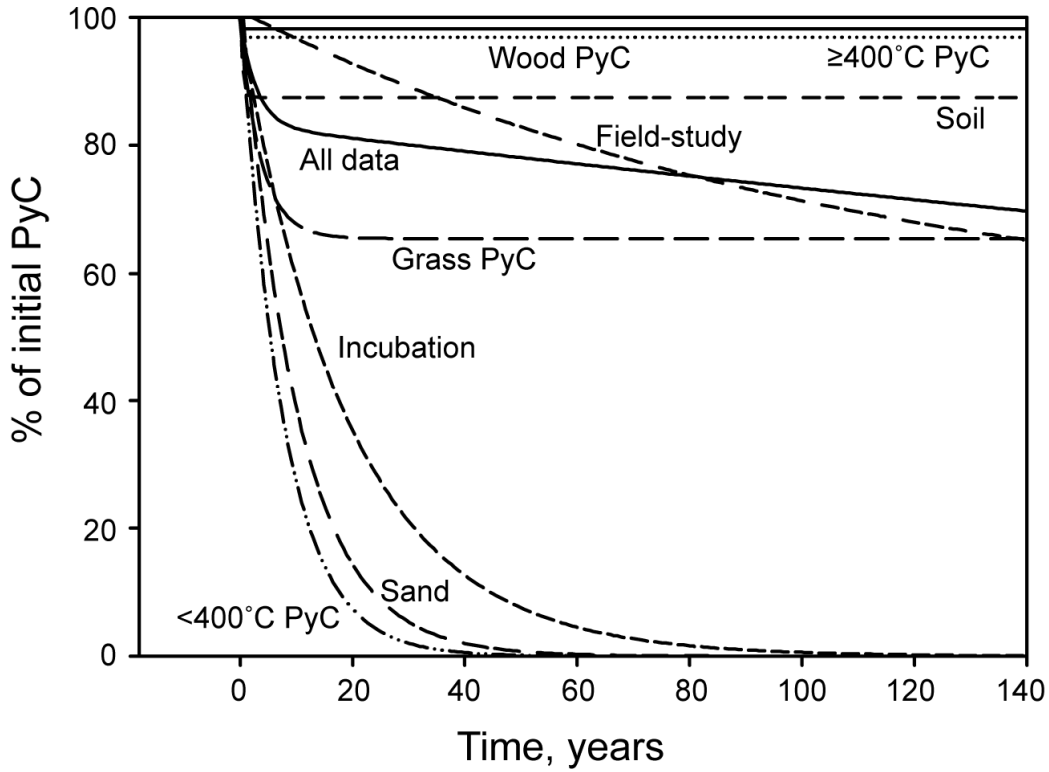
Supplement Table A6: Number of data point for each factor in the study

	Incubation	Field study	Grass PyC	Wood PyC	<400ºC pyrolysis temperature	≥400ºC pyrolysis temperature	Sand medium	Soil medium
Incubation	**47**	0	22	22	22	25	31	16
Field study	0	**6**	0	3	0	1	0	6
Grass PyC	22	0	**22**	0	10	12	12	10
Wood PyC	22	0	0	**22**	9	13	19	3
<400ºC pyrolysis temperature	22	0	10	9	**22**	0	13	9
≥400ºC pyrolysis temperature	25	1	12	14	0	**26**	18	8
Sand	31	0	12	19	13	18	**31**	0
Soil	16	6	10	3	9	8	0	**17**

Supplement Table A7: Turnover time calculated for grouped factors using one pool model, either average of individual study or model fit using non-linear regression.

Grouped factors	One pool	
	Average	Model fit
Incubation study (n = 47)	τ = 55 y; Stdv.[1] = 58	τ = 18 y; RSME[2] = 7.8
Field Study (n = 6)	τ = 353 y; Stdv.[1] = 249	τ = 300 y; RSME[2] = 11.2
Grass PyC (n = 22)	τ = 37 y; Stdv.[1] = 41	τ = 12 y; RSME[2] =10.1
Wood PyC (n = 22)	τ = 79 y; Stdv.[1] = 50	τ = 64 y; RSME[2] =2.8
<400°C Pyrolysis temperature (n = 22)	τ = 25 y; Stdv.[1] = 26	τ = 8 y; RSME[2] = 8.6
≥400°C Pyrolysis temperature (n = 26)	τ = 81y ; Stdv.[1] = 51	τ = 79 y; RSME[2] = 3.0
Quartz sand (n = 36)	τ = 73 y; Stdv.[1] = 48	τ = 20 y; RSME[2] = 8.5
Soil medium (n = 17, excluding field studies)	τ = 23 y; Stdv.[1] = 33	τ = 19 y; RSME[2] = 5.6

Note: [1] standard deviation; [2] root mean square error



Supplementary Figure A1: Two-pool double exponential model on grouped data namely, (a) Incubation studies (C_{fast} = 46 %, r^2 = 0.16); (b) Field Study(C_{fast} = 49.8 %, r^2 = 0.51); (c) Grass PyC* (C_{fast}= 46%, r^2 = 0.23); (d) Wood PyC* (C_{fast} = 6%, r^2 = 0.03); (e) <400° C PyC*(C_{fast}= 49.8%, r^2 = 0.46); (f) ≥400° C PyC* (C_{fast} = 46%, r^2=0.23); (g) Quartz Sand medium* (C_{fast} = 50%, r^2 = 0.20); (h) Soil medium* (C_{fast} = 12%, r^2 = 0.34). (* denotes only incubation studies included).

References

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally, altered Pinus resinosa (Red Pine) wood. *Organic Geochemistry,* **33**, 1093-1109.

Bird MI, Moyo C, Veenendaal EM, Lloyd J, Frost P (1999) Stability of elemental carbon in a savanna soil. *Global Biogeochemical Cycles,* **13**, 923-932.

Brodowski S (2005) Origin, function, and reactivity of black carbon in the arable soil environment. *PhD Thesis, Institut für Bodenkunde, Bonn*, 183.

Bruun EW, Hauggaard-Nielsen H, Ibrahim N, Egsgaard H, Ambus P, Jensen PA, Dam-Johansen K (2011a) Influence of fast pyrolysis temperature on biochar labile fraction and short-term carbon loss in a loamy soil. *Biomass & Bioenergy,* **35**, 1182-1189.

Bruun S, Jensen ES, Jensen LS (2008) Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. *Organic Geochemistry,* **39**, 839-845.

Cheng CH, Lehmann J (2009) Ageing of black carbon along a temperature gradient. *Chemosphere,* **75**, 1021-1027.

Cheng CH, Lehmann J, Engelhard MH (2008a) Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochimica Et Cosmochimica Acta,* **72**, 1598-1610.

Cheng CH, Lehmann J, Thies JE, Burton SD (2008b) Stability of black carbon in soils across a climatic gradient. *Journal of Geophysical Research-Biogeosciences,* **113**, -.

Hamer U, Marschner B, Brodowski S, Amelung W (2004) Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry,* **35**, 823-830.

Hammes K, Torn MS, Lapenas AG, Schmidt MWI (2008) Centennial black carbon turnover observed in a Russian steppe soil. *Biogeosciences,* **5**, 1339-1350.

Hilscher A, Heister K, Siewert C, Knicker H (2009) Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. *Organic Geochemistry,* **40**, 332-342.

Hilscher A, Knicker H (2011a) Carbon and nitrogen degradation on molecular scale of grass-derived pyrogenic organic material during 28 months of incubation in soil. *Soil Biology & Biochemistry,* **43**, 261-270.

Hilscher A, Knicker H (2011b) Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28 month microcosm experiment. *Organic Geochemistry,* **42**, 42-54.

Kuzyakov Y, Subbotina I, Chen HQ, Bogomolova I, Xu XL (2009) Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. *Soil Biology & Biochemistry,* **41**, 210-219.

Lehmann J (2007) Bio-energy in the black. *Frontiers in Ecology and the Environment,* **5**, 381-387.

Lehmann J, Skjemstad J, Sohi S *et al.* (2008) Australian climate-carbon cycle feedback reduced by soil black carbon. *Nature Geoscience,* **1**, 832-835.
Liang B, Lehmann J, Solomon D *et al.* (2008) Stability of biomass-derived black carbon in soils. *Geochimica Et Cosmochimica Acta,* **72**, 6069-6078.
Liang BQ, Lehmann J, Sohi SP *et al.* (2010) Black carbon affects the cycling of non-black carbon in soil. *Organic Geochemistry,* **41**, 206-213.
Major J, Lehmann J, Rondon M, Goodale C (2009a) Fate of soil-applied black carbon: downward migration, leaching and soil respiration. *Global Change Biology,* **16**, 1366-1379.
Nguyen BT, Lehmann J, Kinyangi J, Smernik R, Riha SJ, Engelhard MH (2008) Long-term black carbon dynamics in cultivated soil. *Biogeochemistry,* **89**, 295-308.
Nocentini C, Certini G, Knicker H, Francioso O, Rumpel C (2010) Nature and reactivity of charcoal produced and added to soil during wildfire are particle-size dependent. *Organic Geochemistry,* **41**, 682-689.
Singh N, S. A, Schmidt MWI (2010) Mechanisms of charcoal degradation during its initial stages of decomposition. In: *European Geosciences Union Meeting.* pp Page, Vienna, Austria, Copernicus.
Vasilyeva NA, Abiven S, Milanovskiy EY, Hilf M, Rizhkov OV, Schmidt MWI (2011) Pyrogenic carbon quantity and quality unchanged after 55 years of organic matter depletion in a Chernozem. *Soil Biology & Biochemistry,* **43**, 1985-1988.
Zimmerman AR (2010) Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science & Technology,* **44**, 1295-1301.

Supplementary material, Manuscript II

Supplement Table A8: Characteristics of soil in each mesocosms after 10 months in 0-5 cm depth.

Sl No.	Treatments	Plot name	CEC mmol kg $^{-1}$ soil	pH	C	H g kg^{-1} soil	N	$\delta^{13}C$ ‰ V-PDB	$\delta^{15}N$ ‰ air
1	Wood, N+	Plot B	79.80	5.7	48.3 (0.7)	10.6 (0.6)	3.32 (0.1)	-4.92	25.35
2	Wood, N0	Plot B	40.25	4.7	22.7 (0.4)	7.4 (0.1)	1.76 (0.1)	51.19	150.46
3	Wood, N+	Plot C	92.90	5.8	51.4 (0.8)	11.1 (0.7)	3.42 (0.2)	9.76	46.81
4	Wood, N0	Plot C	82.72	5.9	40.5 (1.0)	10.4 (0.1)	2.99 (0.1)	20.09	78.01
5	Wood, N+	Plot D	75.14	6.3	43.1 (1.3)	0.97 (0.1)	2.76 (0.0)	-3.65	20.97
6	Wood, N0	Plot D	77.96	6.3	25.2 (2.4)	8.7 (0.4)	2.07 (0.1)	-12.69	82.30
7	PyOM, N+	Plot B	89.54	6.2	46.1 (3.2)	10.7 (0.2)	2.90 (0.1)	97.18	205.23
8	PyOM, N0	Plot B	60.37	4.6	32.2 (1.4)	8.4 (0.5)	2.18 (0.1)	114.75	191.75
9	PyOM, N+	Plot C	83.13	5.7	49.3 (1.9)	10.5 (0.8)	3.08 (0.1)	94.88	199.92
10	PyOM, N0	Plot C	77.85	5.9	50.4 (1.6)	10.4 (0.4)	2.99 (0.0)	114.73	255.73
11	PyOM, N+	Plot D	63.93	5.7	39.2 (0.6)	8.5 (0.2)	2.34 (0.1)	168.72	336.01
12	PyOM, N0	Plot D	48.40	5.2	35.2 (1.0)	8.0 (0.2)	2.24 (0.1)	124.99	269.18
13	Control, N+	Plot B	74.55	5.9	39.5 (0.5)	9.8 (0.4)	2.94 (0.2)	-27.08	-2.12
14	Control, N0	Plot B	91.44	6.4	29.1 (0.6)	8.9 (0.6)	2.23 (0.1)	-29.98	-0.92
15	Control, N+	Plot C	61.79	5.9	36.4 (0.6)	8.3 (0.2)	2.67 (0.1)	-27.24	-1.88
16	Control, N0	Plot C	65.56	5.9	40.0 (1.5)	9.5 (0.4)	2.88 (0.2)	-26.22	-2.08
17	Control, N+	Plot D	68.06	5.6	31.3 (0.7)	8.7 (0.4)	2.42 (0.1)	-26.40	-1.33
18	Control, N0	Plot D	65.83	5.5	28.2 (0.3)	8.6 (0.2)	2.22 (0.1)	-26.43	-0.93

Supplementary material, Manuscript III

Microbial community structure at family rank changes in PyOM amended temperate forest soil after one year

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Supplement Table A9: The DNA concentration in each soil sample with description of the barcode sequence and linker primer used for PCR.

Sl no.	Sample description	Sample ID	Primers	Barcode sequence	Linker Primer Sequence	DNA concentration	
						ng/ul	25ng/ul
1	Wood N+	95a	Universal	GTTGACGACAGC	CCGTCAATTCCTTTRAGTTT	4.31	5.80
2	Wood N+	95	Universal	TACGTGTACGTG	CCGTCAATTCCTTTRAGTTT	3.8	6.58
3	Wood N+	68	Universal	TAGATCCTCGAT	CCGTCAATTCCTTTRAGTTT	33.45	0.75
4	Wood N+	51	Universal	TCGATGAACTCG	CCGTCAATTCCTTTRAGTTT	5.43	4.60
5	Wood N0	96	Universal	TAGCGACATCTG	CCGTCAATTCCTTTRAGTTT	3.51	7.12
6	Wood N0	42	Universal	TAACTCTGATGC	CCGTCAATTCCTTTRAGTTT	15.54	1.61
7	Wood N0	58	Universal	TAGCCTCTCTGC	CCGTCAATTCCTTTRAGTTT	6.59	3.79
8	Wood N0	58a	Universal	TATCGCGCGATA	CCGTCAATTCCTTTRAGTTT	4.3	5.81
9	PyOM, N+	83	Universal	TCACTTCTCGCT	CCGTCAATTCCTTTRAGTTT	16.97	1.47
10	PyOM, N+	65	Universal	TACTAATCTGCG	CCGTCAATTCCTTTRAGTTT	19.18	1.30

11	PyOM, N+	63a	Universal	GTGCAATCGACG	CCGTCAATTCCTTTRAGTTT	5.96	4.19
12	PyOM, N0	78	Universal	TATCTCGAACTG	CCGTCAATTCCTTTRAGTTT	10.59	2.36
13	PyOM, N0	43	Universal	TATCAGGTGTGC	CCGTCAATTCCTTTRAGTTT	13.51	1.85
14	PyOM, N0	53	Universal	TCACTGGCAGTA	CCGTCAATTCCTTTRAGTTT	6.97	3.59
15	Control, N+	81	Universal	TCCACGTCGTCT	CCGTCAATTCCTTTRAGTTT	19.22	1.30
16	Control, N+	72	Universal	TACGCGCTGAGA	CCGTCAATTCCTTTRAGTTT	4.21	5.94
17	Control, N+	72a	Universal	TAGCATCGTGGT	CCGTCAATTCCTTTRAGTTT	21.88	1.14
18	Control, N+	75	Universal	TACTACATGGTC	CCGTCAATTCCTTTRAGTTT	11.84	2.11
19	Control, N0	93	Universal	TCGATACTTGTG	CCGTCAATTCCTTTRAGTTT	14.22	1.76
20	Control, N0	44	Universal	TAAGCGCAGCAC	CCGTCAATTCCTTTRAGTTT	7.55	3.31
21	Control, N0	47	Universal	TCATGGTACACT	CCGTCAATTCCTTTRAGTTT	6.51	3.84

Supplement Table A10: Analysis of variance in the relative abundance at family rank due to organic amendment and N treatments.

Sl no.	Phylum	Class	Order	Family	Organic Input	N	Organic Input * N
1	*Acidobacteria*	*Acidobacteria-5*			0.215	**0.012**	0.125
2	*Acidobacteria*	*Chloracidobacteria*			0.609	**0.021**	0.575
3	*Acidobacteria*	*Holophagae*	*Holophagales*	*Holophagaceae*	0.203	0.261	**0.026**
4	*Actinobacteria*	*Actinobacteria*	*Other*	*Other*	0.183	**0.015**	0.697
5	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*		0.705	**0.066**	0.552
6	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Actinosynnemataceae*	0.557	**0.034**	0.120
7	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Dermacoccaceae*	**0.018**	**0.055**	**0.018**
8	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Frankiaceae*	0.103	**0.012**	0.907

9	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Kineosporiaceae*	0.652	0.396	**0.026**
10	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Microbacteriaceae*	**0.020**	0.601	**0.002**
11	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Micrococcaceae*	0.102	0.200	**0.040**
12	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Nakamurellaceae*	0.363	**0.033**	0.378
13	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Streptomycetaceae*	0.364	**0.034**	0.398
14	*Actinobacteria*	*Actinobacteria*	*Actinomycetales*	*Streptosporangiaceae*	**0.063**	**0.041**	**0.040**
15	*Actinobacteria*	*Actinobacteria*	*Solirubrobacterales*	*Solirubrobacteraceae*	0.898	0.251	**0.047**
16	*Bacteroidetes*	*Sphingobacteria*	*Sphingobacteriales*	*Sphingobacteriaceae*	**0.024**	0.606	0.544
17	*Chlorobi*	*SM1B09*			0.680	**0.038**	0.609
18	*Cyanobacteria*	*Chloroplast*	*Stramenopiles*		0.178	0.329	**0.026**
19	*Elusimicrobia*	*Elusimicrobia*	*MVP-88*		**0.037**	0.681	0.147
20	*Firmicutes*	*Clostridia*	*Clostridiales*	*Clostridiaceae*	0.343	**0.046**	0.586
21	*Firmicutes*	*Clostridia*	*Clostridiales*	*Veillonellaceae*	0.884	**0.029**	0.795
22	*Gemmatimonadetes*	*Gemmatimonadetes*	*Other*	*Other*	**0.040**	**0.092**	**0.041**
23	*Proteobacteria*	*Alphaproteobacteria*	*Rhizobiales*	*Hyphomicrobiaceae*	0.443	**0.050**	**0.084**
24	*Proteobacteria*	*Alphaproteobacteria*	*Sphingomonadales*		0.886	**0.036**	0.857
25	*Proteobacteria*	*Betaproteobacteria*			0.398	**0.010**	0.108
26	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*		0.480	**0.044**	0.457
27	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Alcaligenaceae*	0.362	0.531	**0.046**
28	*Proteobacteria*	*Betaproteobacteria*	*Burkholderiales*	*Comamonadaceae*	0.221	**0.009**	0.300

29	*Proteobacteria*	*Betaproteobacteria*	*Nitrosomonadales*	*Nitrosomonadaceae*	0.808	**0.037**	0.329
30	*Proteobacteria*	*Betaproteobacteria*	*Rhodocyclales*	*Rhodocyclaceae*	0.203	**0.045**	0.247
31	*Proteobacteria*	*Deltaproteobacteria*	*Bdellovibrionales*	*Bacteriovoracaceae*	0.416	**0.036**	**0.053**
32	*Proteobacteria*	*Deltaproteobacteria*	*CTD005-82B-02*		0.155	**0.012**	**0.007**
33	*Proteobacteria*	*Deltaproteobacteria*	*Myxococcales*	*Nannocystaceae*	0.354	0.404	**0.037**
34	*Proteobacteria*	*Gammaproteobacteria*	*Xanthomonadales*	*Xanthomonadaceae*	**0.013**	**0.000**	0.247
35	*SC4*				0.103	0.552	**0.041**
36	*Verrucomicrobia*	*Opitutae*	*Opitutales*		0.392	**0.014**	0.447
37	*Verrucomicrobia*	*Spartobacteria*	*Spartobacteriales*	*Spartobacteriaceae*	0.275	**0.028**	0.160

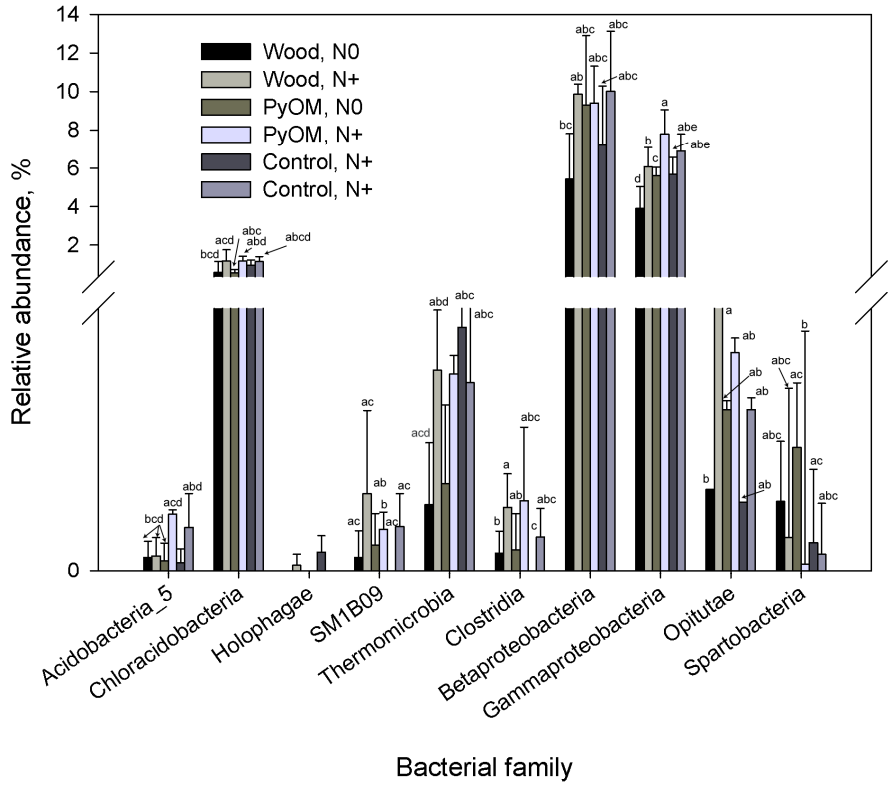
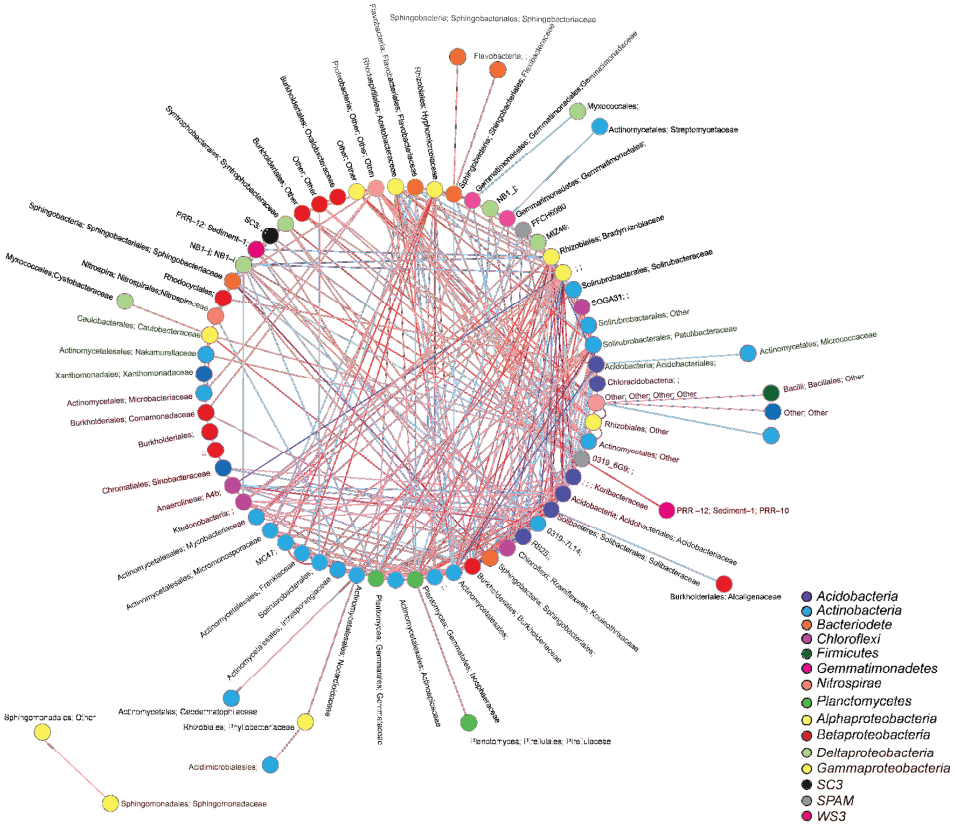


Figure A2: Relative abundance of bacterial community affected significantly either due to organic input or added N. For PyOM-amended soil, added N increased the relative abundance of family *Acidobacteria-5* and *Chloracidobacteria* (*Acidobacteria*). In the wood amended soil, added N resulted in the increase in the relative abundance of all bacterial class in the above figure, except *Acidobactera-5*, *Holophage* and *Clostridia*. The difference in the letters above bars represents significance difference ($p<0.05$). The error bar is standards error (n=3).



Supplement Figure A3: Network of co-occurring families due to added N effect. Different colors of the circles correspond to phyla. The colors of the lines denote the strength and sign of the correlations: blue colors for negative, red colors for positive. These specific network edges have correlations > 0.7 and p-value < 0.001.The line segment have 3 shades of blue, and 3 shades of red with darker shades denoting higher (in absolute value) correlations binned into 3 regularly spaced bins from 0.7 to 1 (from -0.7 to -1 for negatives).

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Publications of the Master thesis

Singh, Nimisha; and Turner, Andrew; (2009) Leaching of copper and zinc from spent antifouling paint particles. *Environmental pollution* 157, 371-376.

Singh, Nimisha; and Turner, Andrew; (2009), Trace metals in antifouling paint particles and their heterogeneous contamination of coastal sediments. *Marine Pollution Bulletin,* 58, 555-564.

Turner, Andrew; Singh, Nimisha; and Millard, Leigh, (2008) Bioaccessibility and bioavailability of Cu and Zn in sediment contaminated by antifouling paint residues. *Environment science and technology* 42, 8740-8746.

Turner, Andrew; Singh, Nimisha; and Richards, Jonathan P.; (2009) Bioaccessibility of metals in soils and dusts contaminated by antifouling paint particles. *Environment Pollution* 157, 1526-1532.

How can I stand on the ground every day
and not feel its power?
How can I live my life stepping on this stuff
and not wonder at it?
William Bryant Logan

(Dirt: The Ecstatic Skin of the Earth)